Factors influencing the efficiency of two parasitoids of the potato tuber moth (PTM)

by

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TO MY FAMILY

ESPECIALLY TO MY FATHER

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DECLARATION

I hereby declare that the work described in this thesis 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 due reference is made in the text.

I consent to the thesis being made available for loan or photocopying.

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PUBLICATIONS

Two papers, containing some results from this thesis, have been published in the conference proceedings:

1) Salehi, L. and Keller, M. A. (1995). The response of the parasitic wasp *Orgilus lepidus* to local variation in the density of its host, *phthorimaea operculella* Zeller. *Proceedings of the 26th Annual General Meeting and Scientific Conference of Australian Entomological Society. Tamworth Centre for Crop Improvement, New South Wales, Australia, 23-28 September.* P. 40.

2) Salehi, L. and Keller, M. A. (1996). Functional response and competition between the two parasitoids of the potato tuber moth. *Proceedings of the First Joint Scientific Conference of New Zealand and Australian Entomological Societies. Lincoln University, Canterburg, New Zealand. 22-27 August.* P. 60.

3) Salehi, L. and Keller, M. A. (1997). Host discrimination by two parasitoids of the potato tuber moth. *Proceedings of the 28th Annual General Meeting and Scientific Conference of Australian Entomological Society. The University of Melbourne, 28th September-30th 3Rd October.* P. 84.

SUMMARY

Apanteles subandinus Blanchard and *Orgilus lepidus* Muesebeck are two braconid, solitary endoparasitoids of the potato tuber moth (PTM), *Phthorimaea operculella* Zeller. They have been introduced into Australia and are established in different potato growing regions, but previously there were no data about factors influencing their efficiency. This project aimed to fill that gap and measure aspects of their biology and ecology related to their efficiency as successful biological control agents of PTM, especially in augmentative control programs.

Laboratory and field experiments measured responses to temperature, reproductive ability, searching behaviour, spatial density dependence, functional response to host density, competition and host discrimination of the two parasitoids. The studies were conducted with larvae of PTM infesting either potato tubers or foliage. The results were compared for the two parasitoid species. Subsequent studies attempted to determine which of these factors influence the efficiency of these two parasitoids in controlling PTM.

Development Egg-to-adult development time was shorter for *A. subandinus* than *O. lepidus* at five constant temperatures, and both were shorter than that of PTM. The development rate of the two parasitoids and PTM was slowest at 15°C and fastest at 35°C; however, mortality was 40% for *A. subandinus* and 84% for *O. lepidus* at 35°C. Adult longevity of males of both parasitoids was shorter than that of females. Longevity of *A. subandinus* was longer than that of *O. lepidus* at 15°C and 35°C. Conversely, longevity of *O. lepidus* was longer at 20, 25 and 30°C. These results indicated that *A. subandinus* has greater temperature tolerance than *O. lepidus*.

Reproduction The effects of host density, plant tissue, exposure time and temperature on the reproductive activity of the parasitoids were studied. The number of eggs laid by female *O. lepidus* at high PTM larval density (160/plant) was significantly higher than that at low larval density (20/plant). There was a relationship between the longevity of female wasps and the total number of eggs laid during their lifespan. Those with high oviposition rates died sooner than those with lower oviposition rates. This trend was similar for *A. subandinus*, but there were no significant differences between oviposition rates at different densities for this species. The total number of eggs laid daily by both parasitoids increased with increasing periods of exposure to PTM larvae. The optimal temperature for oviposition was 25°C for *O. lepidus* and 30°C for *A. subandinus*. For *O. lepidus*, there was no difference between the number of eggs laid during 24hr on established hosts on tubers and on foliage, whereas for *A. subandinus* less oviposition occurred in hosts established on tubers. Counting the eggs in the ovaries of female wasps determined that the number of eggs produced in the ovarioles was higher for *O. lepidus* than for *A. subandinus* during their lifetime, regardless of whether they had been exposed to hosts. Females of *O. lepidus* laid more eggs than those of *A. subandinus* under all conditions measured in these experiments. In the laboratory, fecundity ranged from 90-224 for *O. lepidus* and 70-158 for *A. subandinus*.

The results of these experiments indicated that there is no pre-oviposition period in females of either species. *O. lepidus* has the advantages of being larger, has larger ovaries and produces more eggs than *A. subandinus*. The latter species has the advantage over *O. lepidus* of a shorter developmental time. Oviposition by both species is influenced by host density and temperature. The proportion of males of both species was lower in the field than for insects reared in the laboratory.

Host finding The results from flight tunnel choice tests indicated that females of *A. subandinus* females responded more strongly to plants with host feeding damage than to mechanically damaged plants. When given a choice between an infested potato plant and an infested eggplant or tomato plant, *O. lepidus* landed more frequently on the infested potato plant. By contrast, *A. subandinus* females were attracted equally to all plants with PTM.

Spatial density-dependence To investigate the rate of parasitism at various host densities, experiments were conducted in an open wind tunnel and in the field. The results indicated that the number of hosts parasitised by *O. lepidus* and *A. subandinus* increased as the host density increased. However, at all host densities the percentage of parasitism by *O. lepidus* was higher than those of *A. subandinus.* Both field and laboratory experiments indicated an attraction of *O. lepidus* and *A. subandinus* females to patches with high host density, and the overall results showed a spatial density-dependent mortality of PTM.

Functional response The functional responses of *A. subandinus* and *O. lepidus* were studied to evaluate the effectiveness of these species in reducing PTM populations. In each experiment there were four patches, each with a different density replicated four times. The mean of the recorded data from four patches indicated a Type 1 functional response for *O. lepidus* and Type 2 response for *A. subandinus* (Holling, 1959). These result confirmed the data on density dependent experiments of the two parasitoids.

Competition To test the hypothesis that competition between *A. subandinus* and *O. lepidus* would influence coexistence on PTM, both a laboratory and a field experiment were carried out and the mechanisms of competition were identified. The development of embryos and their morphological changes were also observed to facilitate identification of each species in multiparasitised hosts. The results of these experiments clarified that the two parasitoids

compete, not at the adult stage, but at the first instar larval stage. *O. lepidus* has higher fecundity and dominates *A. subandinus* in this larval competition. However, the total percentage of parasitism was higher when the parasitoids occured in combination than when they operated alone. The results of laboratory and field experiments indicated that *O. lepidus* was always competitively superior to *A. subandinus* in both reproductive potential and larval competitive ability.

Host-discrimination The behaviour of *O. lepidus* and *A. subandinus* females was studied in a flight tunnel when they were exposed to previously self-, conspecific- and interspecific-parasitised hosts. There was superparasitism by both parasitoids when they encountered hosts parasitised by self and conspecific. Most of the superparasitism occurred during the first exposure to wasps suggesting that discrimination is weakest for newly parasitised hosts. *A. subandinus* laid fewer eggs than *O. lepidus* in either the self or conspecific treatments indicating that host discrimination in both cases by *A. subandinus* was greater than *O. lepidus* at less than one hour. Neither species was able to discriminate PTM larvae parasitised by the other. The percentages of super- and multi-parasitism were higher when hosts had mined in thick leaf tissues, such as veins, than when hosts were located in the mesophyll.

Conclusion The results of this study indicate that *O. lepidus* is potentially more useful than *A. subandinus* in controlling PTM under the conditions tested. *O. lepidus* responded positively to increasing host density and parasitised a greater number of hosts during its lifetime than *A. subandinus.* However, *A. subandinus* was more tolerant to temperature extremes than *O. lepidus.* Differences between *A. subandinus* and *O. lepidus* permit their coexistence, and they are complementary in the biological control of PTM. Thus, it is suggested that augmentative releases of both species would be more effective in controlling PTM populations than releasing either species alone.

ABBREVIATIONS

%	Percent
/	Per
X	Magnification
>	More than
<	Less than
°C	Degrees Centigrade
ANOVA	Analysis of variance
cm	Centimetre
D	Dark
ed.	Editor
e.g.	Example given, for example
et al.	And others
g	Gram
hr	hour and hours
i.e.	In example, that is
IPM	Integrated Pest Management
kg	Kilo gram
L	Light
m	Meter
min	minute
mg	Milligram
ml	Millilitre
mm	Millimetre
No., n.	Number

OWT	Open Wind Tunnel
p	Probability
PBS	Phosphate Buffer Saline
PMDS	Pest Model Design System
Rep.	Replicate
PTM	Potato Tuber Moth
Rh	Relative humidity
s	second
sd.	standard of the differences of the means
sem.	standard error of the mean
sp.	Species
temp.	Temperature
var.	variety

Chapter one

An introduction to *Apanteles subandinus* and *Orgilus lepidus* and their host *Phthorimaea operculella* with a general overview of their use in biological control

Potato leaves and tubers damaged by PTM larvae

"Little has been published on factors regulating number of Potato tuber moth a serious pest of potatoes in Australia" G. H. L. Rothschild, 1986

1.1 Introduction

Assessing the efficacy of a parasitoid is important in developing successful biological pest control. In this research a variety of related factors are addressed that influence the ability of the parasitic wasps *Apanteles subandinus* Blanchard and *Orgilus lepidus* Muesebeck to control potato tuber moth (PTM), *Phthorimaea operculella* Zeller.

Potato production is an important industry in Australia, with 1,150,000 tonnes of potatoes produced on 40,000 hectares valued at $349,000,000 in 1993-94 (McLennan, 1996). Yields of potato can be substantially reduced by insect pests, diseases and weeds. More than 30 species of insects have been identified as pests of potato plants in the world, of which more than 20 are found in Australia (Appendix 1.1).

The PTM is host specific to solanaceous plants. It was introduced from the New World in the 18th century and has spread throughout the potato growing regions in all states of Australia (Callan, 1974). The larvae damage potato plants by mining leaves, stems and tubers in the field and feeding on potato tubers in storage. This insect is recognised as the most serious and destructive pest of potatoes in many countries in the world.

Until recently, cultural practices and insecticide applications have been the main approaches to the control of PTM. Attempts at biological control of PTM have been made in Australia mostly with parasitic wasps, including two braconid, solitary, larval endoparasitoids, *A. subandinus* and *O. lepidus*. Both attack the first instar of the PTM (Horne, 1993a). The biological factors that influence the efficiency of these wasps have not been reported. In this context, four main elements are of interest for biological control of PTM.

The first is the searching behaviour of the parasitoids. Searching parasitoids respond to a series of environmental cues, such as semiochemicals, which lead them to appropriate habitats and hosts. Parasitoids are often attracted to the food plant of hosts (Vinson, 1976; Colvin and

Gibson, 1992). Frass or salivary secretions from the host contain chemicals that act as primary stimuli for host location (Lewis *et al.*, 1972; Carde and Bell, 1995; Foster and Harris, 1997).

The second factor is the functional responses and density dependence of the parasitoids. The relationship between host density and rate of parasitism is a central feature of host-parasitoid interactions in nature. Parasitoids may respond to changes in host density in two ways, by functional responses or numerical responses. In the case of a functional response, the rate of oviposition by individual parasitoids increases as host density increases (Solomon, 1949; Holling, 1959a; Hassell, 1978). Functional responses are due to changes in the behaviour of individual parasitoids to changing host densities. In the case of a numerical response, the number of hosts parasitised changes as a result of a change in parasitoid density (Solomon, 1949). Numerical responses arise when natality, mortality and larger scale movements of parasitoids are affected by changing host density. The study of density dependence and functional responses is potentially useful in elucidating the effect of parasitism on an insect population (Holling, 1961).

Thirdly, host discrimination and competition involving both superparasitism and multiparasitism by *A. subandinus* and *O. lepidus* could influence their successful coexistence on PTM and the degree of control they deliver. In this regard, the ability of the female parasitoid to distinguish between unparasitised hosts and hosts previously parasitised by herself (self-host discrimination) or another member of the species (conspecific-host discrimination) should be studied. *O. lepidus* females are known to leave a chemical marker to prevent superparasitism by other females (Greany and Oatman, 1972), but interactions of this type with other parasitoid species attacking PTM (multiparasitism) are unknown. There is no previously published information in this regard for *A. subandinus.*

Competition between parasitoids may occur either among adults, among larvae within the host, or between adult and larval stages. Flanders and Oatman (1987) found that competition among parasitoids of PTM may affect the establishment and distribution of the parasitoid. It is not known, however, whether larval parasitoids of PTM compete within the host.

Lastly, the environmental conditions required for the development of a parasitoid may be more restrictive than those for the host (Vinson, 1981). The influence of temperature on the development, longevity and reproduction of *A. subandinus* and *O. lepidus* has not been elaborated.

The research reported here was focused on the responses to temperature, the reproductive ability, searching behaviour, spatial density dependence, functional response to host density, competition and host discrimination of *A. subandinus* and *O. lepidus*. These studies aimed to determine which of these factors might influence the efficiency of the two parasitoids in controlling PTM. Laboratory and field experiments were conducted to provide information about the above aspects and evaluate the role of the two species in biological control of PTM.

1.2 Potato tuber moth

1.2.1 Distribution

The potato tuber moth is native to the New World and originated from South America (Lloyd, 1972; Sankaran and Girling, 1980). It is now distributed throughout many parts of the world in tropical, sub-tropical, dry and warm areas (Rothschild, 1986). The PTM is considered an important pest in Australia, New Zealand, the United States, Nigeria, India and Pakistan (Lloyd, 1972; Whiteside, 1980; Rothschild, 1986; Kumar and Ballal, 1990; University of California, 1992; Dillard *et al.*, 1993). This wide distribution has been effected through transportation of infested potato tubers. It was introduced into Australia in this way in the late

eighteenth century and is now widespread and abundant in all the potato growing regions in Australia (Callan, 1974; Hamilton, 1985; Briese, 1986; Horne, 1990a; Dillard *et al.*, 1993).

1.2.2 Morphology

The adult of *P. operculella* is a greyish-brown moth with a wingspan of 12-16 mm. The egg is oval and about 0.5mm in diameter. The larva is about 1mm long and pale in colour when newly emerged, and 12 mm long and greenish- or pinkish-grey at the end of the final 4th instar. The pupa is dark brown and about 8mm long (Povolny, 1991), (Fig. 1.1), (Appendix 1.2).

1.2.3 Biology

The life cycle of the PTM varies in different regions of the world. Environmental factors such as host plant quality, temperature and moisture influence the development and productivity of PTM. The PTM is a cosmopolitan insect over a wide climatic range between latitudes 17°N and 43°S (Briese, 1986). Temperature is the main determinant of the rates of key processes in the life system of this insect. The moth distribution is positively correlated with temperature (Trivedi *et al.*, 1994). The lower developmental threshold temperature has been variously determined to be between 9.5 and 13.7°C, and the optimum temperature between 24 and 29°C in different parts of the world (Appendix 1.3). Whiteside (1980) identified a strong negative correlation between rainfall and numbers of potato moths. Females do not lay their eggs on moist substrates (Langford and Cory, 1932; Traynier, 1975; Fenemore, 1978), and mortality of neonate larvae increases when soil moisture exceeds 10% (Foot, 1979). The optimal relative humidity for PTM has been estimated at 60-70% (Finney *et al.*, 1947; Rahalkar *et al.*, 1985).

The life-cycle of PTM is completed in about a month when temperatures are warm and there may be several generations per year. PTM has two generations in areas that have short

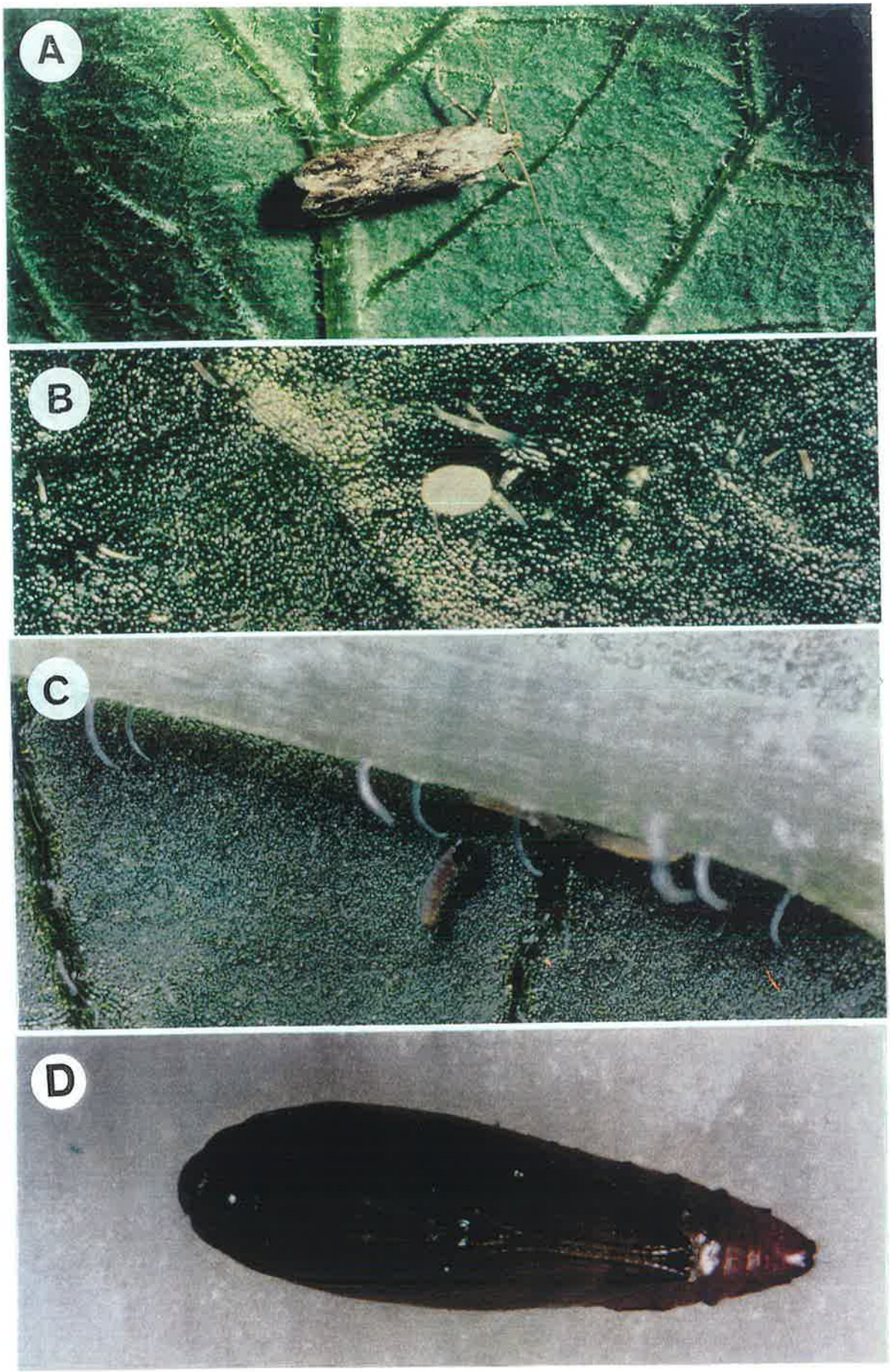


Figure 1.1: Potato tuber moth (PTM), *Phthorimaea operculella*, adult (**A**), egg (**B**), larva (**C**) and pupa (**D**).

cropping seasons, but under warm and dry conditions it has more than ten generations (Clausen, 1978; Kroschel and Koch, 1994). In areas where there is a severe winter, hibernation occurs in the larval or pupal stages (Sankaran and Girling, 1980). Breeding in stored potatoes can go on throughout the year except at low temperatures. The duration of adult mating ranges from 25min to 4hr. Each female deposits an average of 104 eggs during her lifetime under laboratory conditions (Kabir, 1994).

1.2.4 Host plants

The PTM is largely restricted to feeding on plants of the Solanaceae. Within this family at least 40 host species have been identified, particularly in the genus *Solanum* which includes the potato, *Solanum tuberosum* L. (Attia and Matter, 1939; Foot, 1976). More than 20 species of host plants of PTM have been identified in Australia (Atherton, 1936; Lloyd, 1943; Anon, 1958; Champ, 1966; Callan, 1967; Briese, 1980) and most belong to the Solanaceae (Appendix 1.4). Five of these hosts are crops (potato, tomato, tobacco, eggplant and bell pepper), and potato is a particularly favourable host plant for PTM (Kroschel and Koch, 1994). Contact stimuli on the surface of potato plants elicit significantly stronger oviposition responses than other plants in host selection experiments (Varela and Bernays, 1988).

The larvae of PTM feed on the mesophyll, forming blotchy mines in potato leaves. Sometimes the larva burrows into the petiole and continues its way into the stem, from which it reaches the tuber. The larvae commonly penetrate tubers near the buds and bore shallow galleries under the skin. As the density of larvae increases, they go deeper into the tuber. Some researchers (e.g. Broodryk, 1971) have reported a tuber infestation of up to 45% under field conditions. In addition to the direct loss of quality in infested tubers which are no longer marketable, the pest is transmitted in tubers to the potato store, where breeding and infestation of tubers continues if they are not in cold storage.

1.3 Management of PTM

A variety of methods are used by farmers around the world to control PTM. Chemical control of this pest is unsatisfactory because PTM larvae penetrate inside the foliage and tubers, but cultural and biological methods have proved their worth, and merit wider application (Horne, 1993).

1.3.1 Cultural control

Cultural practices for control of PTM include tillage, hilling-up, irrigation, variation of planting depth and planting date, use of resistant varieties of potato, removal of haulms before harvesting and removal of self sown potatoes and weeds before planting, especially *Solanum nigrum* L., blackberry nightshade (Table 1.1).

Irrigation, particularly overhead sprinklers, helps to limit infestations of tubers by preventing cracking of the soil. PTM females cannot access tubers in uncracked soil (Foot, 1974; Ali, 1993). Shelton and Wyman (1979) found that the total infestation under furrow irrigation was over 58 times greater than under sprinkler irrigation where soil cracking was less, but PTM populations in the foliage did not differ between irrigation systems. In New Zealand field trials infestation by PTM was significantly lower in irrigated than unirrigated plots (Nabi, 1984). Similar results were observed by Horne in Australia (personal communication).

Hilling and furrow removal are important in reducing potato tuber infestation in the field. Some potato varieties, such as Kennebec, produce tubers close to the surface of the soil that are accessible to PTM (Bacon, 1960). Planting depth or the use of deep-setting varieties is also important to control PTM activity. Siddig (1988) found that increasing sowing depth from 3 to 7cm reduced damage and increased yield.

Resistant varieties of potato have the advantage of sustaining less damage by PTM. Many potato varieties are resistant to this pest (Raman and Palacios, 1982; Ojero and Mueke, 1985; Ghalla and Chandla, 1986; Arx *et al.*, 1987; Ahmed *et al.*, 1991). Raman *et al.* (1994), increased resistance of potato plants to PTM by crossing between cultivars, and obtained 171 resistant hybrids. Laboratory investigations by Valencia (1984), showed that the preferred cultivar by PTM in Australia was Exton and the least preferred was Red Lasoda. There have been neither field investigations nor practical use of resistant varieties in management of PTM in Australia.

Field cleaning and destruction of infested potato tubers (Sankaran and Girling, 1980) and prompt transfer of harvested potatoes to safe storage is also helpful in reducing damage.

At present, cultural control has not been successful in many regions, but farmers generally employ a combination of these techniques during the year to reduce the economic losses caused by PTM (Horne, 1995, personal communication) (Table 1.1).

1.3.2 Physical control

Irradiation of eggs, pupae and adults to control PTM has been studied on stored potatoes in the laboratory (Rananavare *et al.*, 1989 and 1990). Saour and Makee (1997) found that with 25 Krad irradiation, mating ability, frequency of mating and fertility of the males were decreased, but longevity was not affected. They also observed that a maximum of 91% sterility was induced when they irradiated male pupae of PTM with 45 Krad.

This technique has not been used as a practical method to control PTM, it could not be expanded in practise because it requires more research on both economics and health concerns.

Table 1.1: IPM tactics for the potato tuber moth (e.g.: Callan, 1974; Horne, 1993).

Method	Time	Practice	Reference*
Cultural control	Before planting	1) Removal of self-sown potatoes and weed	Foot, 1974
		2) Tillage	Siddig, 1988
	At planting	1) Deep planting (10 cm) or use of deep-setting varieties	Bacon, 1960; Ali, 1993
		2) Use of resistant varieties	Ahmed *et al.*, 1991; Raman *et al.*, 1994
		3) Early planting	Ali, 1993
	During plant growth	1) Hilling-up to covering tubers (at 6, 10 and 14 weeks)	Bacon, 1960
		2) Irrigation and stopping ground cracking over tubers	Shelton and Wyman, 1979; Nabi, 1984
	At harvesting	1) Early harvest, rapid handling and cold storage	Ali, 1993
Sex pheromones	At emergence of PTM adult	1) Mass trapping to disrupt mating.	Raman, 1984; Sanders, 1989
Biological control	Cropping season	1) Augmentation of natural enemies, especially parasitic wasps.	Watmough *et al.*, 1973; Callan, 1974; Franzmann, 1980; Briese, 1981; Horne, 1993
Chemical control	Only if essential and will not interfere with biological control.	1) Application of suitable insecticides or other agents.	Hilje and Cartin, 1990

* References cited here present only as examples.

1.3.3 Chemical control

Chemical control measures in field crops are generally aimed at killing PTM larvae in potato foliage and haulms to minimise the more serious type of damage caused by larvae attacking tubers in soil prior to harvest, and subsequently in storage. Hilije and Cartin (1990) reported a range of over 32 chemical pesticides used by farmers around the world against PTM and application techniques that can be used at different times during the growing season.

Chemical control has not been consistently successful, possibly due to poor timing (Watmough *et al.,* 1973), and the development of resistant PTM populations (Champ and Shepherd, 1965). In addition, chemical treatment of many crops damaged by PTM, such as tobacco and tomato, is limited or not permitted over a large part of the vegetative period (Izhevskive, 1986). Then, lack of effectiveness, resistance and legislation with chemical control of PTM necessitate the development of a biological control program.

1.3.4 Sex pheromones

Using pheromones of PTM for various purposes began when Adeesan *et al.* (1969) found a gland in the last abdominal segment of the moth that releases a sex pheromone. Researchers (e.g. Raman, 1984) have used sex pheromones for monitoring, mass trapping and mating disruption of PTM on potatoes in the field and in storage. To address the increasing problem of insecticide resistance in PTM, pheromones could be used in two principal ways, trapping and mating disruption (Sanders, 1989). The pheromone of PTM is commercially produced and used in many countries of the world. This chemical is also used as a lure in traps to monitor PTM in the field but it is unsuitable for control of pest populations (Raman, 1984).

1.3.5 Biological control

Biological control of a destructive pest is a major alternative to the use of pesticides in agriculture. As a technology, it is over 100 years old (Waage, 1996). Classical biological control has proved to be an economical and environmentally benign solution to many severe pest problems (Greathead, 1995).

Attempts at biological control of PTM have been made in at least 20 countries, mostly with parasitoids shipped by the Commonwealth Institute of Biological Control (CIBC) (Callan, 1974). However, the efficiency of these parasitoids is not yet sufficiently understood. It is necessary to identify efficient and effective natural enemies of PTM, particularly parasitoid species for mass rearing and augmentative releases to reduce crop damage as part of an integrated pest management (IPM) program. Biological control of PTM began in Australia in 1943, when introductions of exotic parasitoids were attempted (Wilson, 1961); a second attempt was made in the 1960s (Callan, 1974; Franzmann, 1980; Briese, 1981; Horne, 1993).

1.3.5.1 Parasitoids

PTM has a large complex of parasitoids worldwide, many of which are native polyphagous species that have adopted it as an additional host in areas where the pest has been introduced (Oatman and Platner, 1989). More than 70 species of parasitoids, belonging to 12 families of Hymenoptera, two species of Diptera and one species of Nematoda, have been reported as attacking PTM by researchers worldwide (Appendix 1.5).

Australian entomologists have reported at least 14 parasitoids of PTM from six families of Hymenoptera (Rothschild, 1986). Six species are indigenous and the others have been introduced into Australia (Appendix 1.5). Of these, three species, *A. subandinus*, *O. lepidus* and *Copidosoma desantisi* Annecke & Mynhardt, have become well established in Australia (Callan, 1974; Franzmann, 1980 and Briese, 1981). P. Horne (unpublished data) surveyed the

major parasitoids of PTM in 1994 in different districts of Australia and the relative importance of each parasitoid species. *O. lepidus* was not recorded in New South Wales or Western Australia, but *A. subandinus* was established in all potato growing regions of Australia.

1.3.5.2 Predators

There are many insect predators recorded as major causes of mortality in PTM populations, with more than 20 species of predators having been reported by different researchers in Australia (Appendix 1.6). There is no reports from literature of inundative predator releases to control PTM populations.

1.3.5.3 Pathogens

Special attention is given to mass production of microbial agents in modern classical biological control. Different researchers (e.g. Thomas and Poinar, 1973) have reported species of bacteria, fungi and viruses which cause mortality of larval PTM in the field (Appendix 1.7). Reed (1971) identified a granulosis-type baculovirus (Gv) in Australia and Briese (1980) recorded 10-34% mortality of PTM larvae due to Gv in some potato growing regions in this country. Recently Salama *et al.* (1996) reported an IPM program based on a mixture of *Bacillus thuringiensis* and a granulosis virus. Although pathogens can cause mortality in different stages of PTM, the interactions between PTM and its pathogens require further research.

1.3.6 Integrated pest management (IPM)

IPM is the careful selection and integration of various methods of control (cultural, physical, biological and chemical) at suitable times during the cropping cycle (Van den Bosch and Stem, 1962). The growing recognition of problems associated with the application of

chemical pesticides alone has led to increased attention to IPM (Zalom, 1993). IPM approach is to minimise application in the crop and maximise biological and cultural control in the field and applying pesticide when necessary (Horne, 1991).

Several approaches are available for the development of IPM for PTM. Strategies suggested by researchers for the integrated pest management of PTM are listed in Table 1.1 (Ortu and Floris, 1989; Siddig, 1990; Wahundeniya, 1990; University of California, 1992; Fuglie *et al.*, 1993; Horne, 1993a). The strategies involve changing practices during different seasons and in different conditions. In these approaches the emphasis is given to the identification of cultural practices and biological control agents that allow a reduction of pesticide applications.

1.4 *A. subandinus* and *O. lepidus* (Hymenoptera: Braconidae) as biological control agents of PTM

A. subandinus and *O. lepidus* are specific solitary endoparasitoids. Female wasps of both species oviposit in early instar PTM, and the parasitoid larvae develop in the growing larva until the host is ready to pupate. The parasitoids then kill the host, pupate and emerge as adult wasps. Female *A. subandinus* oviposit in 1-day-old larvae, and *O. lepidus* females prefer 1- to 3-day-old larvae. [IC1]Only a single larva per host completes its development (Cardona and Oatman, 1975, Oatman *et al.*, 1969).

There are some regions where all introduced parasitoid species are present, but not all have established throughout Australia (Briese, 1981). [IC2]Among the exotic parasitoids imported into Australia thirty years ago (see Appendix 1.5), *O. lepidus*, *A. subandinus* and *Copidosoma spp* have become well-established only in some areas of Australia (Horne, 1993). Successful establishment occurs only where favourable environmental conditions are present. Although many factors can prevent establishment and affect the efficiency of parasitoids in a new agro-

ecosystem (DeBach, 1964), there has been no thorough investigation of the effects of environmental conditions on *A. subandinus* and *O. lepidus*.

1.4.1 *Apanteles subandinus* Blanchard

Apanteles is probably the largest microgastrine genus in Australia and is estimated to contain 100-150 species from mainland Australia and Tasmania (Austin and Dangerfield, 1992). *A. subandinus* was first described by Blanchard in 1947 (Cardona and Oatman, 1975). It originated from South America and was exported to different countries of the world. The University of California received it from Peru and Argentina in 1964 (Cardona and Oatman, 1975) and it was shipped from California to Australia between 1964 and 1969. It established more successfully in Australia than in California (Briese 1981, 1986; Callan, 1974). *A. subandinus* is now distributed in all the potato growing regions in Australia (Horne, 1993).

Morphology

Larval *A. subandinus* has three instars. The first instar is mandibulate-caudate and the second and third instars are hymenopteriform (Fig. 1.4). The cocoon is spun by the mature last instar in the prepupal stage beside the host and the pupa is exarate. Male and female adults are black; the length of the female is 3.86mm and of the male 3.63mm (Fig. 1.2). The ovipositor of the female is 0.63mm in length (Appendix 1.2).

Biology

A. subandinus is an arrhenotokous insect; females are diploid and develop from fertilised eggs, whereas males are haploid and develop from unfertilised eggs (Cook, 1993). The biology of this wasp was investigated by Cardona and Oatman (1975) under laboratory conditions. They found that the peak of reproduction of *A. subandinus* was 345 progeny per female at on optimum temperature of 26.5°C. These researchers illustrated the effects of

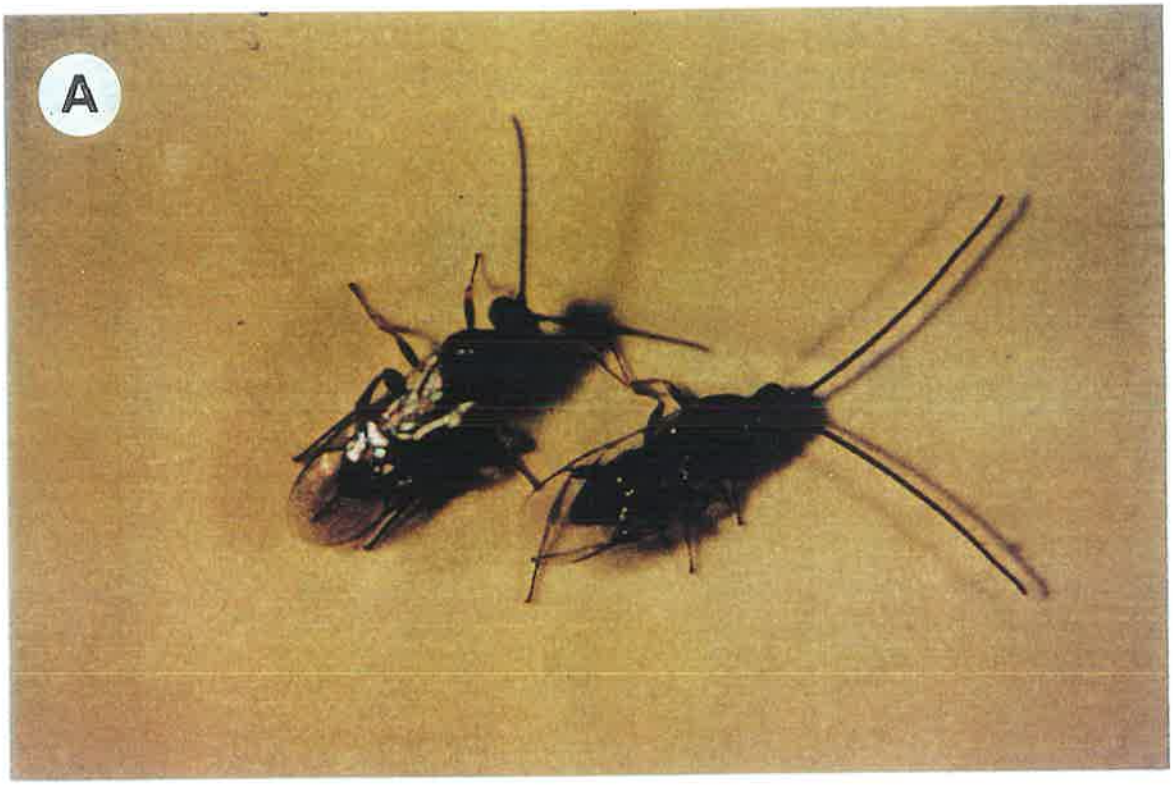


Figure 1.2: *A. subandinus* adults, female (left) and male (right) (**A**), a female (**B**).

temperature on the length of each development stage of the wasp. They found that between 15°C and 32°C speed of development was directly related to temperature (Appendix 1.3), and a constant temperature of 35°C prevented development of the parasitoid beyond the first instar.

1.4.2 *Orgilus lepidus* Muesebeck

O. lepidus was first described by Muesebeck in 1967. This wasp was originally obtained from Argentina and was first exported to California from South America, and then shipped from California to Australia (Oatman *et al.*, 1969). Although *O. lepidus* has not established in California it is well established in Australia (Callan, 1974).

Morphology

Larval *O. lepidus* have three instars. The first instar is mandibulate and the other two are hymenopteriform (Fig. 1.4). The first instar has a developmental rate which varies with that of the host larva, but most parasitised larvae moult to the second instar between six and seven days after oviposition (Oatman *et al.*, 1969). The third instar emerges from the host larva and develops to a prepupa within the host cocoon, inside which it can be seen. The wasp cocoon is spun by the mature last instar inside the host cocoon. Adults are yellowish-brown and the two sexes are grossly similar in appearance, except that the female has a long straight ovipositor which is about the same length as the body from the tip of the head to the end of the abdomen (Fig. 1.3), (Appendix 1.2).

Biology

The biology of *O. lepidus* has been investigated by Oatman *et al.* (1969) under laboratory conditions of 26.7°C and 50% relative humidity (RH). Adults emerge during daylight hours. The male emerges one day before the female. These researchers observed that the longevity

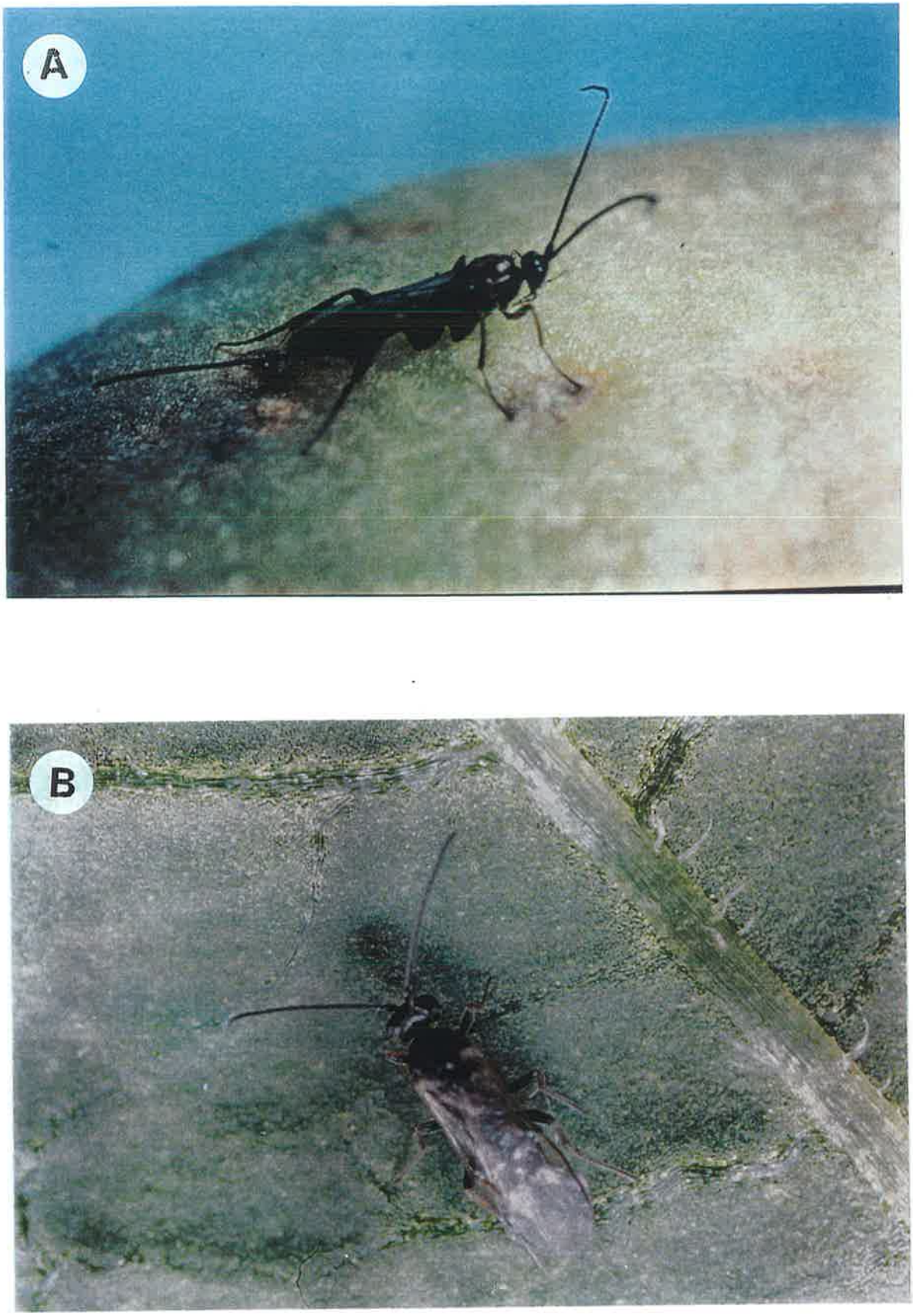


Figure 1.3: *O. lepidus* adults, female (**A**), male (**B**).

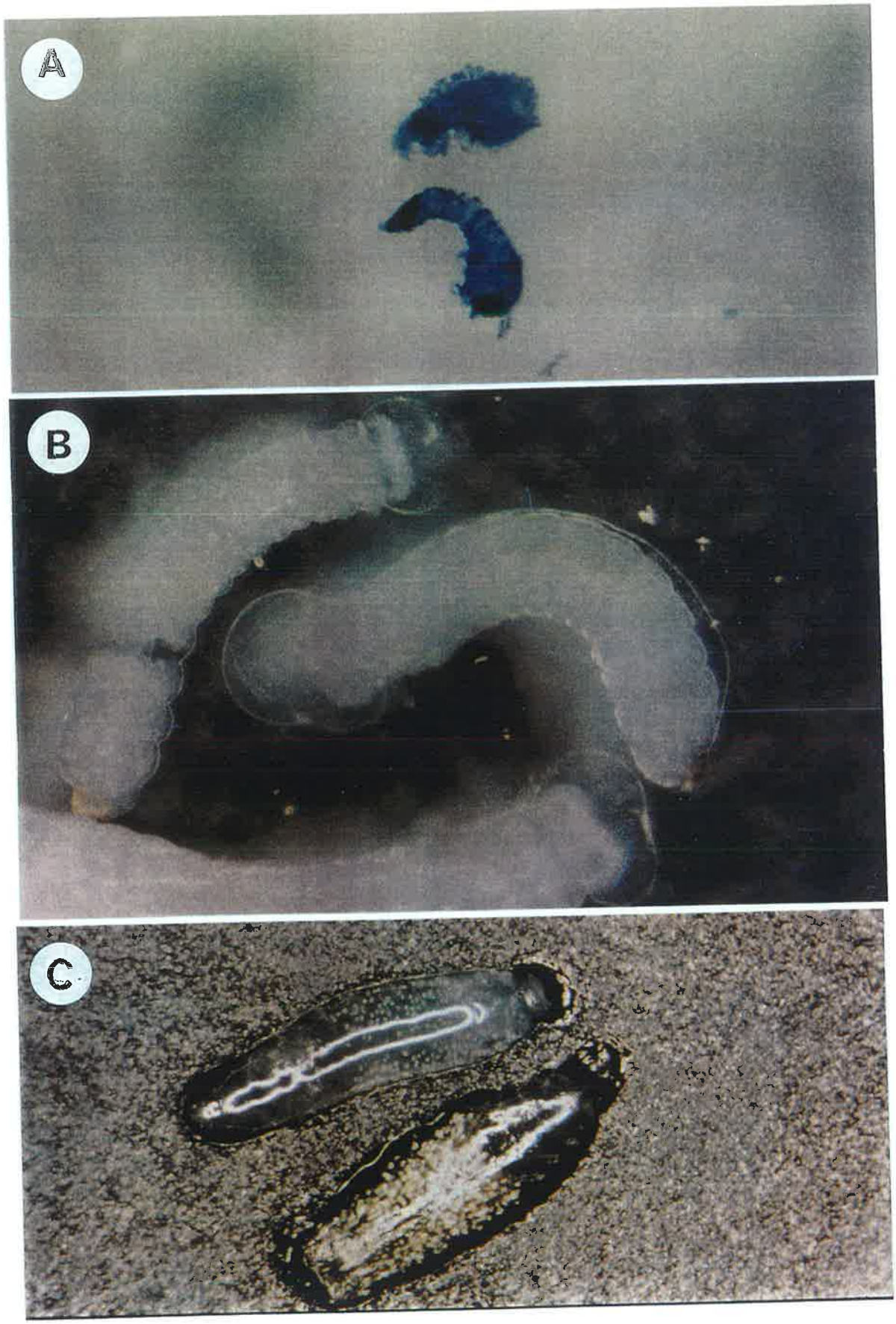


Figure 1.4: First instar larvae of *A. subandinus* (top) and *O. lepidus* (bottom) (**A**), second instar larvae of *O. lepidus* (**B**) and third instar larvae of *A. subandinus* (top) and *O. lepidus* (bottom) (**C**).

of adults is 3-5 days in the absence of food and water, and that parasitoids lived longest, averaging 23.9 days for the male and 21.4 days for the female, when provided with both water and honey. Females can oviposit shortly after copulation. There is no information about the effects of temperature on the development of each stage of this wasp or on its longevity. Female *O. lepidus* average more than 40 progeny per day through the first 10 days of life. A female wasp with an average ovipositional period of 15.7 days produced an average of 632 adult progeny (Oatman *et al.*, 1969).

1.4.3 Factors influencing the behaviour and efficiency of the parasitoids and their host

An evaluation of the characteristics of the dominant parasitoids is important for developing pest management programs. One of the crucial questions is whether the effectiveness of parasitoids can be increased to the extent that they adequately control insect pest populations. Clues to the answer might be obtained by examining the factors that influence or determine the numerical relationships between parasitoid and host populations and the rates of parasitism (Knipling, 1992). For effective biological control of a pest by parasitoids, it can be essential to know their efficiency and searching behaviours (Van Lenteren, 1981). Many factors influence the efficiency of a parasitoid, including the host population structure, environmental conditions and the bioecological behaviour of the species of interest (Huffaker and Messenger, 1976). Further understanding of the biological and behavioural characteristics of the parasitoid are important in a biological control program (Luck, 1990). Although *O. lepidus* and *A. subandinus* head the list of beneficial insects as biological control agents of PTM, no studies have been done on factors influencing the efficiency of these parasitoids, except on host finding by *O. lepidus* (Keller and Horne, 1993). Factors which influence the ability of parasitoids of PTM to be successful biological control agents need to be investigated, so that their beneficial effects can be augmented.

1.4.3.1 Influence of climatic factors

Temperature is recognised as an important factor for the development and mortality of parasitoids and also influences phenology and synchrony of the parasitoid-host relationship (Cave+ and Gaylor, 1988; Miller, 1996). A host may be unsuitable when it occurs in environments which are unfavourable for the development of its parasitoid. Fluctuating temperatures are characteristic of the environments of most insects, yet constant temperatures are most frequently used for laboratory studies of life histories (Hagstrum and Hagstrum, 1970).

[IC3]Briese (1980) demonstrated that the oviposition, fertility, fecundity, adult weight, longevity, duration and survival of the immature stages of PTM are all influenced by temperature. [IC4]Temperature is the main determinant of the rate at which the key processes in the life system of the potato moth proceed (Rothschild, 1986). The success of the two parasitoids depends on the development of PTM at various temperatures.

Temperature responses of *A. subandinus* were studied by Cardona and Oatman (1975). They found that between 15 and 32°C speed of development was directly related to temperature (Appendix 1.2) and a constant temperature of 35°C prevented development of the parasitoid beyond the first instar. These researchers demonstrated that the longevity and reproductive periods of this wasp are inversely related to temperature.

1.4.3.2 Reproductive ability

The reproductive ability of a female parasitoid is determined by its morphological, physiological and anatomical characteristics, its searching behaviour and the environment in which the individual grows into an adult (Thoday, 1961; Minkenberg *et al.*, 1992). In addition, host condition is an important factor for maximum egg-laying of a female parasitoid.

The rate of reproduction of a female wasp is related to host suitability (e.g. host age and size), host density, host location and developmental synchronisation with the host (Van Lenteren, 1986).

In the majority of classical biological control programs, researchers simply release almost all of the suitable natural enemies which happen to be available: they follow a multiple introduction strategy (Huffaker and Messenger 1976).

Classical biological control programmes nearly always depend on a reliable supply of laboratory reared insects (Conlong and Mugoya, 1996). To successfully use parasitoids in biological control, it is necessary to understand the reproductive ability of each parasitoid species to determine how to utilise them properly against a pest.

Information about the reproductive properties of *A. subandinus* and *O. lepidus* and the factors that affect the maximum number of hosts attacked by the Australian populations is sparse. Both species are arrhenotokous with the ability to determine the sex ratio of their offspring. However, the sex ratio of a parasitoid has been shown to be influenced by many factors (King *et al.*, 1995; Wrensch and Ebbert, 1993). To estimate the reproductive activity of the Australian strains of *A. subandinus* and *O. lepidus*, it is necessary to study their egg-laying capacity under different conditions of host density, host plant and temperature.

1.4.3.3 Searching behaviour and host location

Host searching behaviour has been described by many researchers since Salt (1935), and there is a great deal of literature on host acceptance. Laing (1938) hypothesised that parasitoids are guided by a series of chemical and physical cues to the vicinity of their host. Female parasitoids may use physical (e.g. size, shape, texture, movement), physiological and chemical cues (e.g. kairomones) to recognise and parasitise their hosts (Arthur, 1981; Pak, 1986). Doutt (1964) divided the searching behavioural process of a parasitoid into four steps and

Vinson (1975) added the fifth: a) host habitat location, b) host location, c) host acceptance, d) host suitability and e) host regulation.

Many of the cues that influence searching wasps are chemical in nature and originate from the host (kairomones), and from the host's food-plant (synomones) (Lewis and Martin, 1990; Turlings *et al.*, 1990, 1991; Vet and Dicke, 1992; Tumlinson, *et al.*, 1993; Keller and Horne, 1993; Steinberg, *et al.*, 1993 and Takabayashi *et al.*, 1994). The role of kairomones in encouraging parasitoids to search, find and attack their hosts has been studied by many researchers. Lewis *et al.* (1982) found that some parasitoids were attracted to their hosts by chemical odours. Semiochemicals (kairomones and synomones) can be important in increasing the field performance of parasitoids (Lewis, *et al.*, 1975a, 1975b). These chemicals stimulate and prolong searching behaviour of parasitoids and they could be useful in pest management programmes for attracting parasitoids to crops at risk from pest hosts. They could also be used as chemicals prior to release in the field (Gross *et al.*, 1975; Keller and Lewis, 1989).

Chemicals and plant odours appear to play a major role in host location by parasitoids. Ranking of different stimuli will change over the life of the parasitoid; if a parasitoid finds that a certain stimulus is associated with the presence of hosts, its ranking may increase (Lewis *et al.*, 1990; Vet *et al.*, 1995). Thus a naive parasitoid might initially locate a host using a chemical stimulus emitted by the host or even by chance. After finding a host on a particular food plant, it might then learn to use chemicals associated with that food plant in future host location.

Parasitoids often respond to host indicators such as frass or salivary secretions and which contain chemicals acting as primary stimuli for host finding by the parasitoid (Brown *et al.*, 1970; Whittaker and Feeny, 1971). In addition, odours of plants act as cues to indicate host

location and they are attractive to parasitic wasps (Keller and Horne, 1993). Herbivore-damaged leaves produce a C_6 volatile that serves as a synomone for some braconid wasps (Mattacci *et al.*, 1994). This volatile is different from those emitted in response to mechanical damage (Dicke, 1994). In fact, attractive volatiles originate from the plant upon which their host feeds, the host itself and chemicals released as a result of interactions between the host and its food plant (Lewis and Martin, 1990; Steinberg *et al.*, 1993; Du *et al.*, 1996). Plants that have been attacked by herbivores may emit volatile chemicals that are not emitted by undamaged or mechanically damaged plants. This has been shown for several plant species (Dicke *et al.*, 1990a&b; Dicke, 1994; Agelopoulos and Keller, 1994a&b; Du *et al.*, 1996).

Although the role of chemical cues is known to be important in the foraging behaviours of these parasitoids, little information exists about their importance for *O. lepidus* and *A. subandinus*. One study by Keller and Horne (1993) reported that *O. lepidus* can discriminate between the volatiles of a mechanically damaged potato plant and those of a potato damaged by larval PTM.

1.4.3.4 Responses to host density

The ability of a parasitoid to kill proportionately more hosts as absolute host density increases is a desirable attribute for biological control (Huffaker *et al.*, 1971; Van Lenteren and Bakker, 1976). Correlation between host density and rate of parasitism in a patch is a common feature of host-parasitoid interactions in nature (Walde and Murdoch, 1988).

Van den Bosch and Messenger (1973) stated "all natural enemies used in or contributing to successful biological control programs act as density-dependent mortality factors". That is, parasitoids must cause proportionately higher mortality in a high-density population than in a low-density population (Murdoch and Reeve, 1987; Mountford, 1988) to be useful biological control agents. Lessells (1985) investigated how the percentage of hosts parasitised should

vary with initial density in different patches if parasitoids maximise their oviposition rate and compared the theoretical expectations with patterns of parasitism reported in the literature. He made a list of 49 investigations of density-related parasitism, with 17 cases of density dependence (35%), 15 of inverse density dependence (30%), and 17 cases of density independence (35%). Stiling (1987) studied 171 cases of density-related parasitism in insect host-parasitoid systems which were reported in the literature. Among them, the frequency of density dependence was 25%, of inverse density dependence 23% and of density independence 52%. Stiling (1987) found a series of patterns in the incidence of density dependence. Parasitoids which are native, monophagous and solitary should be more density dependent than those without these characters and density dependence is more common among parasitoids which are monophagous, sedentary and have an exposed insect host than others.

Some parasitoids are attracted to patches of high host density by stimuli emanating from the patch, whereas other parasitoids disperse less readily from patches containing a large number of hosts (Waage, 1978). Density-independent or inverse density-dependent aggregation might occur if parasitoids are attracted to certain patches by cues that are either independent or negatively correlated with host density.

1.4.3.5 Functional response

The response of natural enemies to varying density of their host or prey has been extensively studied (e.g. Varley *et al.*, 1973). Several types of responses by parasitoids to their host's density have been described. Solomon (1949) introduced two categories of these responses, as follows:

Functional response: The rate of individual parasitoid attacks changes with changes in host density. It is a behavioural phenomenon (searching).

Numerical response: The parasitoid (or predator) density changes with changes in host density. It is influenced by both behavioural (aggregation) and demographic (reproduction and mortality) properties of natural enemies.

Since Solomon's thesis, many researchers have measured the functional responses of different parasitoids. Holling (1959a&b) worked extensively on the mathematical modelling of functional responses. He identified three types.

1) Type 1 (linear increases): The number of hosts parasitised by a parasitoid increases linearly to a plateau with increasing host density.

$$N_a = aNT_t$$

2) Type 2 (decelerating increasing to a plateau): The number of parasitised hosts increases asymptotically to a plateau with increasing host density.

$$N_a = \frac{aNT_t}{1+aNh_t}$$

3) Type 3 (sigmoid increase): The number of parasitised hosts increases slowly at first and the result is a s-shaped (sigmoid) curve increasing host density.

$$N_a = \frac{bT_t N^2}{1+cN+bT_t h_t N^2}$$

where: N_a is the number of hosts parasitised, N is the number of hosts provided, T_t is the total time, h_t is handling time for each host parasitisation and a, b and c are acceleration constants.

The amount of time spent by a parasitoid in patches with different densities might involve changes of behaviour (Van Alphen and Jervis, 1996). Van Lentern and Bakker (1978)

described two types of time allocation: 1) variable-time and 2) fixed-time. In fixed-time experiments some parasitoids show a Type 2 response (Holling, 1959a), rather than Type 3.

1.4.3.6 Host discrimination

[IC5]The mechanisms by which parasitoids detect and select a suitable host have been widely studied (Salt, 1935; Laing, 1938; Lloyd, 1943; Edwards, 1954; King and Rafai, 1970; Spradberry, 1970; Corbet, 1971; Van Lenteren, 1976; Vinson, 1976, 1981; Strand and Vinson, 1982). Host discrimination is the ability of a parasitoid to distinguish unparasitised hosts from parasitised hosts (Van Lenteren, 1981). [IC6]The ability of insect parasitoids to avoid a host previously parasitised by members of their own species or those of different species has been the subject of several investigations (Vinson, 1991; Van Lenteren, 1981; Charnov and Skinner, 1985; Van Alphen and Vet, 1986). Host discrimination and avoiding superparasitism is not adaptative in all circumstances (Van Alphen *et al.*, 1987). There is much evidence that parasitoids are able to discriminate parasitised hosts, but do not always avoid superparasitism (Van Lenteren, 1981).

Host discrimination prevents wasting eggs, saves search time, serves as a clue for searching among patches (Bakker *et al.*, 1985). [IC7]Host discrimination has not been reported for *A. subandinus*. Greany and Oatman (1972a&b) reported that *O. lepidus* females leave a pheromone to discriminate parasitised hosts.

The extent to which *A. subandinus* and *O. lepidus* distinguish between healthy hosts and those already parasitised is unknown, or if they tend to avoid attacking the latter. The circumstances under which these parasitoids may accept or reject previously parasitised hosts by themselves or other individuals awaits observation and explanation.

1.4.3.7 Competition

The attention of ecologists was focused on competition by Gause in 1934 (DeBach and Sundby, 1963) early in the development of the science. Biological competition occurs whenever two or more individuals of the same species (intraspecific competition) or of two or more species (interspecific competition) are known to utilise a resource that is actually or potentially limited (Clements and Shelford, 1939; Watt, 1965). Some researchers have warned of the negative effects of interspecific competition between introduced natural enemies on pests (Flanders, 1964; DeBach, 1966; Huffaker and Laing, 1972; Ehler and Hall, 1982). [IC8]Interspecific competition among natural enemies of a pest can be of great importance in the application of biological control (Ehler, 1978; Lawton, 1986; Hagvar, 1988; Van Alebeek *et al.*, 1993).

Intra- and interspecific biological competition is usually defined as the active demand by two or more species at the same trophic level for a common resource. Interspecific competition may occur either between adult stages of the parasitoids, between larvae within the host, or between both the adult and larval stages. There are many internal solitary parasitoids, including *O. lepidus* and *A. subandinus*, which have mandibles suitable for fighting in their first instar, but unsuitable for that purpose in the second and third instars (Salt, 1961).

To improve the success rate for classical biological control, a more scientific approach should be adopted in the selection of natural enemies for introduction (Takagi, 1991). In the majority of classical biological control programs, researchers followed the multiple introduction strategy and simply released almost all of the suitable natural enemies which happened to be available. Many biological control workers have believed that introducing all of the available primary parasitoids of an insect pest is the best strategy, since Smith recommended this

approach in 1929. Doutt and DeBach (1964) emphasised that introducing additional species has always increased the effectiveness of host population regulation.

On the other hand some researchers have criticised such an optimistic view and warned of the injurious effects of inter-specific competition between introduced natural enemies. Watt (1965) noted that competition is assumed to occur whenever two species are known to eat the same food species. Ehler and Hall (1982) claimed that a more rational release strategy is needed for modern biological control rather than simply releasing all available species of natural enemies, with the hope that the best species or combination of species will be maintained out in the field. Takagi and Hirose (1994) have achieved successful classical biological control of arrowhead scale using two introduced parasitoids in Japan. They have not supported a policy of disordered release of all of the natural enemies available. These researchers believed that using multiple species of parasitoids for classical biological control requires validation through many more case studies. They reported that a parasitoid species which has the highest reproductive potential with the shortest generation time and another species whose female longevity is longer were the best agents to release together in their chosen system. It is not known whether larval parasitoids of PTM compete within the host. Understanding the interactions of the two parasitoids and the impact of their multiple parasitism on PTM populations is important for a successful biological control program in the field.

In conclusion, effective augmentative biological control of PTM with the parasitoids *A. subandinus* and *O. lepidus* requires an understanding of their efficiency and behavioural ecology and the application of this information for successful control in the field. It is important to investigate the developmental time, egg-laying capacity and host finding ability of the two parasitoids, and also to examine their density-dependence and functional responses

to PTM populations, and their searching behaviour and host discrimination before releasing them together in the field.

1.5 Aims

The aims of this study were to answer the following questions about *A. subandinus* and *O. lepidus*.

1) How do the developmental times of the parasitoids compare to that of their host across a range of temperatures, and how does temperature affect their longevity? How might the effects of temperature influence interaction between the parasitoids and PTM?

Although previous researchers have undertaken studies on these insects, no work has been done using consistent methodology on the Australian strains. It is important to determine this information to assess the potential for establishment of parasitoids in different climatic regions. In addition, the results of this study will enable more efficient mass rearing of these parasitoids for future inundative releases.

2) What is the fecundity of the two parasitoids compared to that of PTM, and under what conditions does the maximum level of parasitism occur?

For a biological control agent to be effective, it is desirable for its reproductive capacity to be high relative to that of the host. An understanding of the relative fecundities of the parasitoids and their host will provide insight into the ability of the parasitoids to manage host populations.

3) What factors influence the host-finding ability of females of *O. lepidus* and *A. subandinus*?

An understanding of the host-finding behaviour of the two parasitoids may play an important part in developing a more effective biological control program for PTM by using infochemicals.

4) Do the parasitoids aggregate in response to high densities of PTM? Parasitoids that aggregate to high densities of their host have a high potential for management of host populations. Investigation of a parasitoid's response to host density has implications for effective decisions for biological control programs, especially those which involve augmentation of natural enemies.

5) Can the parasitoids discriminate among hosts of different categories (unparasitised, self-, conspecific- and interspecific-parasitised)? How does this affect competition between the wasps and levels of parasitism?

The ability of the parasitoids to manage host populations is often reliant on the status of the host. Host discrimination enables parasitoids to search efficiently for hosts and oviposit in hosts where their offspring have the greatest chance of survival.

Inter- and intra-specific competition between parasitoids will influence their ability to manage host populations. Knowledge of positive or negative competition between parasitoids will enable a more informed decision about the release of the parasitoids.

6) Which species should be released to optimise augmentative biological control of PTM?

By understanding all aspects of the interactions between *A. subandinus* and *O. lepidus*, the host, and the environment, the factors that influence the efficiency of the two parasitoids can be determined.

The information provided by this study will evaluate the role of these parasitoids as biological control agents of PTM.

Chapter Two

General materials and methods

Laboratory and field experiments, pheromone trap (left), OWT (centre), established field cages (right).

"Progress in entomological research and the success of pest management programmes depend on our ability to rear insects and establish colonies in the laboratory"

P. Singh, 1985

2.1 Introduction

This chapter describes the general materials and methods used in this research program for experiments that were carried out in the field, wind tunnel and insectary.

2.2 Culture of PTM and the parasitoids

A stock culture of insects (*Phthorimaea operculella, Apanteles subandinus* and *Orgilus lepidus*) was maintained to provide a source of insects of high viability and reproductive capacity for use in experiments at different times. The insectary culture was renewed at the beginning of each summer and autumn with new adult moths and wasps collected either from the field in the Adelaide Hills or Virginia, South Australia, or from near Melbourne, Victoria by Dr Paul Horne (Institute for Horticultural Development, Knoxfield, Victoria). Introduction of fresh field material aimed to prevent inbreeding; in particular, the rearing of many generations of *A. subandinus* in the laboratory increases in the proportion of males.

Methods of rearing PTM and its parasitoids in the insectary have been described by several authors (Finney *et al.*, 1947; Platner and Oatman, 1968 and 1972; Oatman *et al.*, 1969; Rahalkar *et al.*, 1985; Keller and Horne, 1993). A modification of the methods described by Finney *et al.* (1947) was used in this study. Most of the changes made were related to saving time and rearing insects more economically.

Oviposition unit

The PTM oviposition unit (Fig. 2.1.A) consisted of a 450ml matt plastic container with a tightly fitting organdie cover. A vial (20ml) inside the container was filled with 10% honey in water and closed with a lid through which an absorbent cotton roll (35mm long) protruded. Finally, tissue paper was placed on top of the organdie and the container was closed with its plastic lid. Adult PTM were removed from the culture daily using an aspirator connected to a

suction pump and introduced into the oviposition unit through a 10mm hole in its base. The female PTM laid their eggs on the underside of the tissue paper beneath the lid. Eggs were collected daily and the tissue paper replaced.

Egg conservation and hatching

For long term storage (up to 6 weeks), PTM eggs were placed in a cylindrical plastic container (70ml) and refrigerated at 10°C until needed for rearing. When required, the egg container was removed from the refrigerator and placed in a water-filled tray to prevent larvae escaping. Because newly emerged PTM larvae are very small and move very quickly, a thin white cotton cloth was used to seal the lid. Eggs hatched after two days at 24°C in the culture room.

Larval feeding and pupation unit

The insect rearing unit was a closed plastic box (30 × 20 × 7cm) containing egg-sized potato tubers (var. Kennebec or Colliban). Egg-sized potato tubers were used for larval feeding because PTM larvae feed on the surface of tubers and a small tuber provides a greater surface area than a large one (Finney *et al.*, 1947). The box was ventilated through wire mesh in its lid, and a 10mm hole was provided for a water supply (Fig. 2.1.B). The potatoes provided in the rearing unit were not punctured as suggested by Finney *et al.* (1947), because of the results of a preliminary experiment conducted as described below.

Potato feeding trial

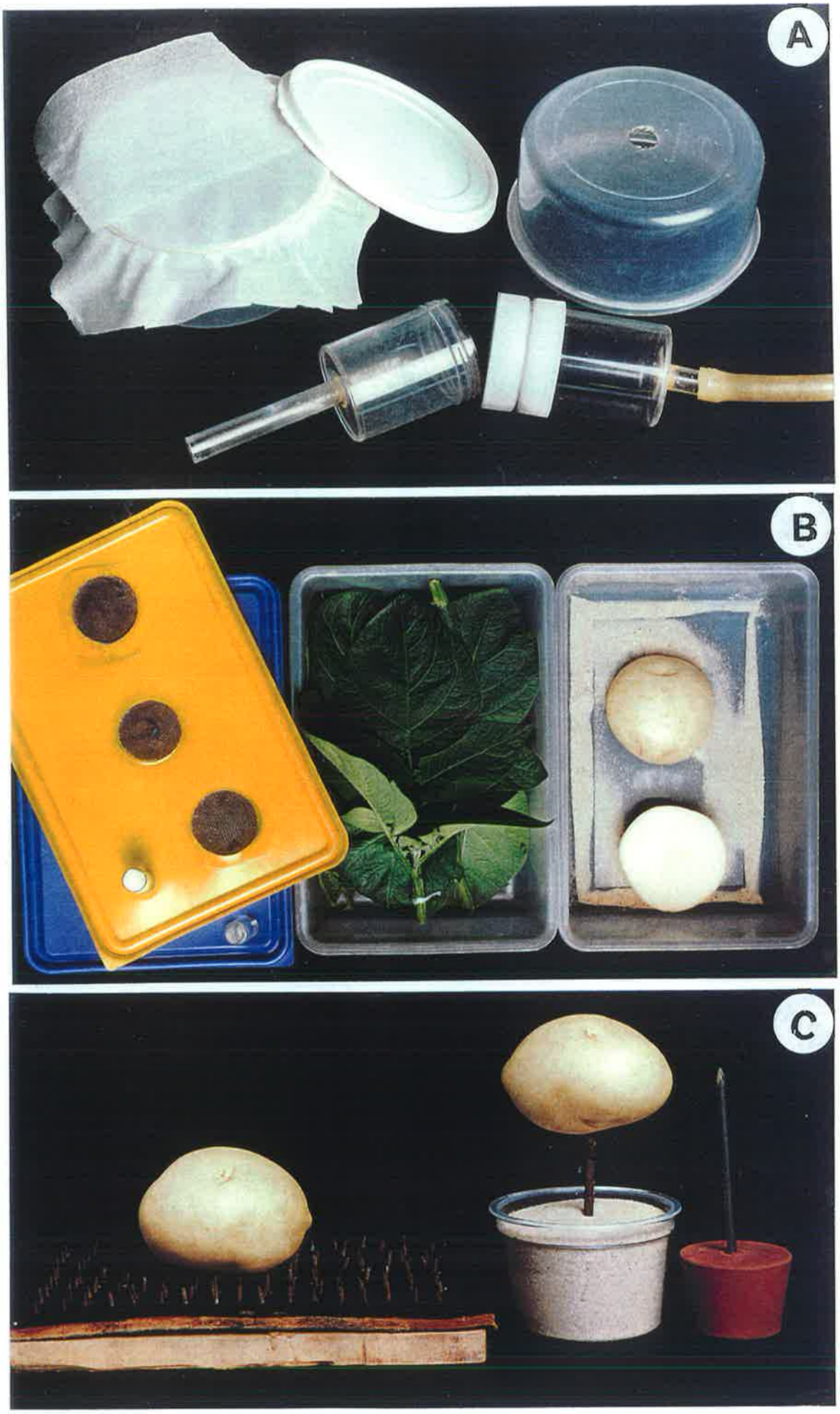
To investigate whether punctured potato tubers are more accessible for larvae and their establishment, two groups of 20 potato tubers, approximately the same size, shape and weight, were selected. Potatoes in the first group were punctured with a tack-studded board (Finney *et al.*, 1947) and those in the second group were left unpunctured (Fig. 2.1.C). Each tuber

Figure 2.1: Rearing equipment:

A) PTM oviposition unit used for routine insectary production of eggs. A 450ml translucent plastic container with tissue paper on top of a tightly fitting organdie cover (top), aspirator connected to a suction pump (bottom).

B) Parasitoid rearing unit containing potato tubers, leaves and white sand.

C) A tack-studded board with potato tuber (left), a potato tuber fixed on a nail driven through a rubber stopper and placed in plastic containers filled with sand (right).


A
B
C

(punctured or intact) was infested with 10 newly emerged PTM larvae using a fine brush. The infested tubers were placed in separate plastic containers (450ml) provided with white sand on the bottom for pupation of larvae. After adults emerged, the number of moths was recorded. The data obtained from this experiment were analysed with ANOVA using Genstat 5 (Lane and Payne, 1996), and there was no significant difference between the two treatments (p = 0.84) (Figure 2.2).

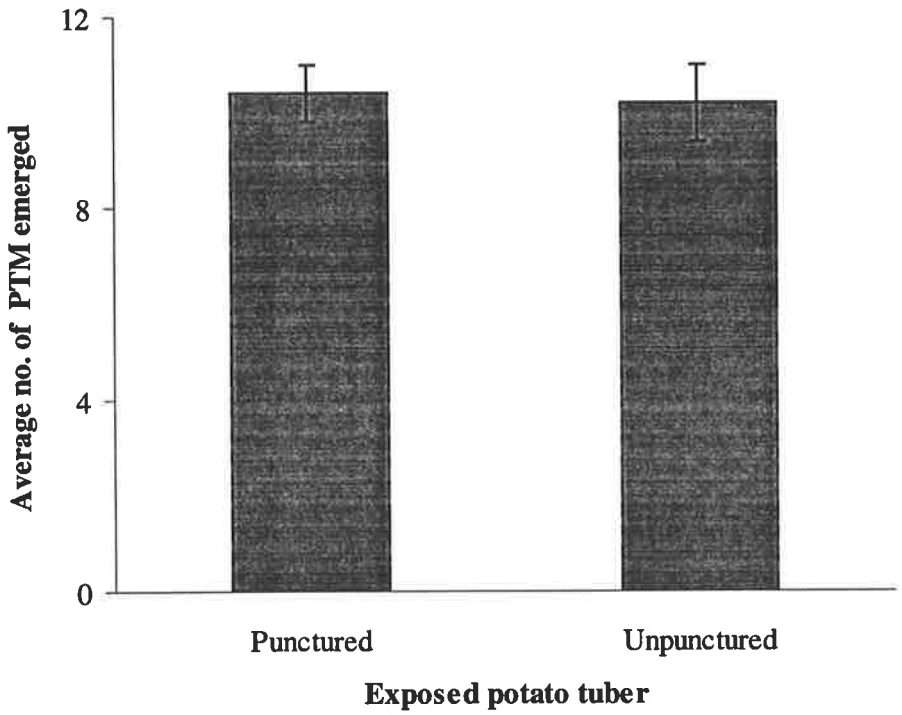


Figure 2.2: Number of PTM larvae emerged from rearing on punctured and unpunctured potato tubers.

It seems that when tubers were punctured they oozed fluid exudate which made a scab. This prevented larvae from penetrating and the leaked ooze killed the larvae. These results do not agree with the finding of Platner and Oatman (1968), who suggested that puncturing potato tubers leads to maximum production of PTM and potato utilisation.

Parasitisation unit

Newly emerged PTM larvae were placed on potato tubers using a fine brush (100 larvae on an egg-sized tuber). The infested potato tubers were fixed on the points of 10cm nails driven through a rubber stopper (size 1.5) and placed in clear plastic containers (150ml) filled with white sand (Fig. 2.1.C). The infested tubers were placed in cages (20 × 16 × 16cm), and female wasps were released in the cages to parasitise the larvae.

Tubers were exposed to *A. subandinus* females 12-24hr after infestation, and to *O. lepidus* females 24-48hr after infestation. The reason for this difference relates to the difference in the length of the females' ovipositors. Although the females of both species can drill potato plant tissues, neither can thrust their ovipositor through tuber tissues, even the most delicate. They insert their ovipositor through the tunnels on the potato tubers made by the PTM larvae. *A. subandinus* females with short ovipositors can parasitise only larvae close to the surface of the tubers. After two days, the tubers were removed and transferred into the larval feeding and pupation unit (described above).

2.3 Host plant growth

Potted plants

For some experiments, potato plants (var. Colliban) were grown from tubers (seed potatoes). Two to three weeks prior to the start of each experiment, potatoes were planted 3-5cm deep in black plastic pots (15 and 20cm in diameter). The soil was University of California mix (U.

C. mix), Waite version (Matkin and Chandler, 1957). The plants were watered 2-3 times a week as necessary. The plants were grown in a glasshouse under natural light and at a temperature of 20-25°C until they reached the height required for the particular experiment.

For wind tunnel experiments, potato tuber buds were planted instead of whole tubers. Buds (2×2cm) were cut from the tubers with a cork-borer; from one tuber (100g) at least five buds could be obtained. Buds were placed in a solution of 2 ppm gibberelic acid water for 15min. The buds were placed on tissue paper until dried and planted 1.5-2cm deep in small plastic pots (10cm in diameter). The buds were then grown in the glasshouse and after 3 weeks had enough foliage to be used in an experiment.

Potato shoots

In some laboratory experiments potato shoots called "container plants" were used (Fig. 2.3.A). A shoot here refers to a potato stem including 4-5 mature leaves. It was possible to cut many shoots 10-15cm high from one pot plant. One day prior to the start of the experiment, suitable shoots were carefully cut as near to the base of auxiliary stems as possible, using a clean sharp razor blade. To keep each shoot fresh for at least seven days, its stem was submerged in water through a hole (10mm in diameter) in the lid of a 150ml container containing tap water. A small cotton wool ball was used to cushion the stem against the side of the hole and to hold the plant upright.

The prepared container plants were then infested with PTM larvae using a fine hair brush. The infested shoots were placed inside a large cage which consisted of a tray (2-3cm deep) filled with tap water to prevent larvae crawling from one container to another. The temperature inside the insectary was maintained at 24°C. Three cool white 40 watt fluorescent tubes one meter in length were maintained to provide artificial lighting during 14:10 LD photophase. The relative humidity ranged between 55 and 65 percent.

For some experiments, during the crop growing season, pest- and disease-free potato shoots were obtained from potato fields. This method was economical and gave quick access to host plants. However these cuttings were not transferred to the glasshouse. The varieties Cristal and Colliban, which have straight stems, proved to be best for fixing in experimental containers.

2.4 Open Wind Tunnel (OWT)

The open wind tunnel (OWT) is a microhabitat with facilities for controlling wind speed, designed by M. A. Keller in 1994 for carrying out experiments on parasitoid behaviour. The OWT is in an ordinary room in the insectary building on the Waite campus of The University of Adelaide. The room (1.8 × 2.2 × 2.8m) has a sliding door and its window is covered with aluminium foil. The walls are coated with cream matt paint which is not attractive to wasps. The room was maintained at a constant environment of 24°C and 50 - 70% RH, and a light level of 8000 Lux was maintained with 4 cool white 40 watt fluorescent tubes one meter in length hung from the ceiling with four chain cords.

The base of the OWT (see Fig. 2.3.A) is a frame made from 2.5 × 2.5cm perforated steel angles with a wooden board (64 × 140cm) fixed over it. Slots (7cm in diameter) are cut in the board 15cm apart in three rows (5 slots in a row). The base is covered with natural soil (1cm deep) and a nylon sheet is fixed 20cm below the wooden board to collect soil which falls through the slots. Two vertical wooden frames covered with terylene voil (54 × 80cm) are fitted to the ends of the base frame. In one of them a fan is fixed 15cm above the base. At the other end a honeycomb cardboard panel is fixed in the wooden frame. The fan is connected to the panel by a polyethylene tunnel (150cm in circumference) for air recirculation.

Wind speed is controlled by a key fixed on the fan. Wind speed was measured with a Compuflow Thermo-Anemometer GGA-65P, ALNOR CO., Finland. Wind speed in the

experimental arena was set on dial number 2 for experiments and the average wind velocity measured at three heights above the soil (10, 20 and 30cm) was 22.4cm/s (Appendix 2.1).

2.5 Wasp handling

For some experiments, wasps were released in the field and their behaviour observed. Because of the limited time available for these experiments, it was necessary to use females experienced prior to their release in the experiment arena, as recommended by Samson-Boshuizen *et al.* (1974), Van Lenteren (1976), and Vet and Dicke (1992).

The releasing cage in the field experiment was a cylindrical 4-litre clear container covered with two layers of nylon organdie(Fig. 2.3.B&C). In the first layer many 10mm holes were cut to allow the wasps to fly out gradually when the outer layer was removed (Fig. 2.3.C). The inner [IC1]organdie layer was attached to the container with Blue Tack. A cotton ball saturated with honey water solution was placed on the base of the cage to provide nourishment for the wasps. In addition, an infested plant was supplied which led to more rapid flight from the container.

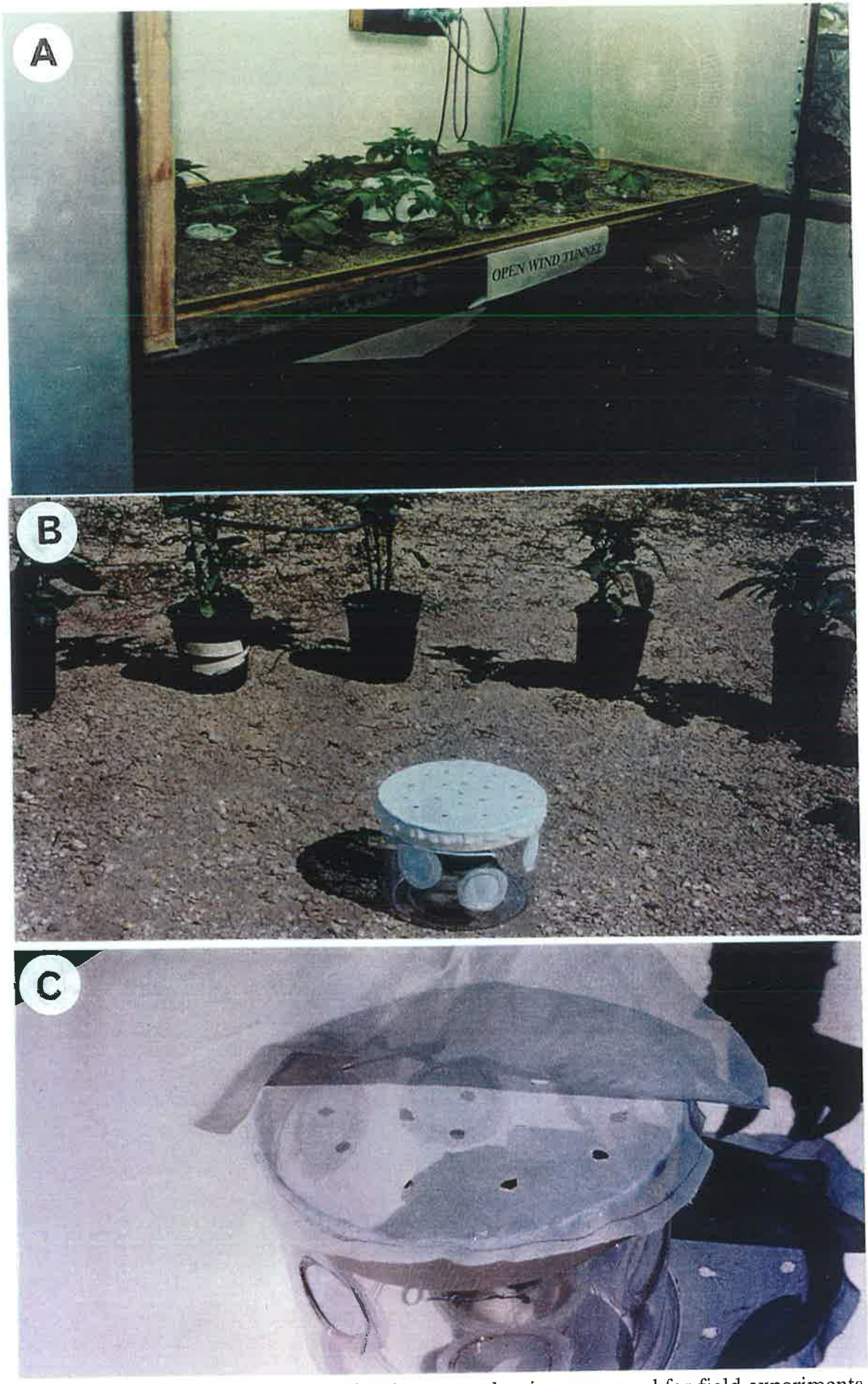


Figure 2.3: The Open Wind Tunnel (A), the wasp releasing cage used for field experiments (B&C

Chapter Three

The influence of temperature on development and longevity of *A. subandinus* and *O. lepidus*

Rearing incubators

"In insects, as in other ectothermic organisms, development rate changes with the temperature of the environment" F. Taylor, 1981

3.1 Introduction

Among the abiotic factors influencing parasitoids and their hosts in the field, temperature has a great effect on parasitoid longevity and rate of development (Taylor, 1981; Cardona and Oatman, 1975; Rothschild, 1986). Information about the influence of temperature on the rate of development and longevity of parasitoids is useful in evaluating their efficiency in the biological control of a pest in the field (Van Driesche, 1983). It is also important for laboratory mass rearing of parasitoids. The temperature-dependent developmental rate curve of an insect is a fundamental feature of its life history (Taylor, 1981). The relationship between developmental time and temperature can be described by both linear and non-linear models, and such models have an important role in IPM research (see Wagner *et al.*, 1984; Logan, 1988; Logan and Weber, 1991; Horne and Horne, 1991).

The relationship between temperature and development of *A. subandinus* and *O. lepidus* was of particular interest because they are now mass reared and distributed for the control of PTM in Australia (Paul Horne, personal communication). The only studies on the effects of temperature on these species were carried out by Cardona and Oatman (1975), using a laboratory strain of *A. subandinus* that had been derived from potato fields in the locality of Ayacucho, Argentina, and by Oatman *et al.* (1969) who described the biology of *O. lepidus* at 26.7°C. While preliminary research has been completed on the distribution of these parasitoids in potato growing regions in Australia (Rothschild, 1986; Horne, 1993), little attention has been given to their temperature-dependent development. Establishment of these species in different potato growing regions in Australia confirms that they have coped with a range of climates in this country.

The life cycle of PTM as a cosmopolitan insect has been investigated in many countries. From the work of Langford and Cory (1932), Briese (1980) and Kroschel and Koch (1994), it

is clear that temperature, particularly early in the season plays an important role in determining the level of infestation during the growing season. This is unlike the winter, or off season, when it appears that food supply becomes more critical (see Rothschild, 1986 for review).

3.1.1 Aims

The aims of this study were:

1) To assess the influence of selected constant temperatures on the developmental times and survival of the life stages of the two parasitoids relative to those of their host;

2) to determine the effects of different constant temperatures on the longevity of the adult wasps.

3.2 Material and methods

To address the above objectives a series of experiments were conducted at five constant temperatures: 15°C, 20°C, 25°C, 30°C and 35±0.5°C, under 24hr light and 50% RH. Temperatures lower than 15°C were not used because PTM larvae all die at 10°C (Markosyan, 1993). In my own pilot test, 10 caged infested tubers each with unparasitised and parasitised PTM larvae were incubated at 10°C; no larvae were found on the tubers after 45 days.

The effects of temperature on the developmental times of egg-larvae, prepupae and pupae of the two parasitoids was investigated; the longevity of the adults that emerged at each temperature was determined; in addition, longevity of adults that developed at 24°C and which were immediately transferred into the various temperatures was determined and finally the developmental time of PTM reared simultaneously with the two parasitoids was determined.

Parasitoid development

In each replicate, 10 newly emerged 1-day-old PTM larvae were transferred to a potato tuber that was approximately 50g in size (Fig. 2.1.C in Section 2.2). After 24hr, the infested tubers were exposed to the parasitoids, one parasitoid and one tuber in each cage (20 × 16 × 16cm) in the culture room (24°C). The tubers were removed after 8hr exposure time and placed in a rearing cage (Fig. 3.1). Each rearing cage consisted of two plastic petri dishes measuring 14 cm in diameter × 4 cm deep. The top dish had a ventilation hole covered with wire mesh and a tube sealed with cotton wool to supply honey and tap water for the emerging adults. White sand was scattered on the floor of the cage to facilitate pupation. The cages were placed in incubators at five constant temperatures and were held at each temperature until all the parasitoid adults emerged. An infested tuber with the same number and age of PTM larvae but not exposed to parasitoids was kept as a control at each temperature. This was done to show firstly, the rate of establishment of PTM on each tuber and secondly, the duration of development for the three stages of PTM.

Cages were inspected daily for evidence of prepupation. In each rearing cage, prepupae were numbered individually with self-adhesive labels on the bottom of the petri dish (Fig. 3.1), and their developmental stages were recorded. From the prepupal stage onward, observations were made twice daily at 12 hour intervals and fresh water was supplied each day to feed newly emerged adults. Both parasitoids always emerge from the final instar of PTM. The parasitised larvae could not be distinguished from normal PTM larvae. The parasitoid could only be recognised and counted at the pupal stage. The mortality of parasitoids and their host was estimated at the pupal stage in each temperature.

Ten caged tubers were used at each of 5 temperatures for each parasitoid species. The relationship between developmental rate and temperature was analysed using the PMDS (Pest

Model Design System) program, described by Logan and Weber (1991). This program indicated the best fitting models for the relationship between temperature and median rate of development for the three life stages of the two parasitoids. The program selects the best model from eight functional relationships between temperature and the median rate of development and provides a graphical display and simulation analysis (Logan, 1988).

Models of temperature dependent development

The equations of the five models suggested by the PMDS program which were fitted to the data and their parameters are as follows:

Exponential model: [$r(T) = \psi \cdot e^{PT}$], where ψ is the minimum rate of development and ρ is the slope of a regression line (Logan, 1988). The two parameters, ψ and P are given by the PMDS program as p1 and p2 respectively (Table in description of Fig. 3.2).

Logan-1 model: $[r(T) = \psi(e^{PT} - e^{P\tau})]$, where τ is estimated from the equation: $\tau = (T_{max} - T)/\Delta T$ and T is in degrees above an arbitrary base temperature (T_{min}), T_{max} is the maximum observed temperature and $\Delta T = T_{max} - T_{min}$ (Logan, 1988).

Stinner model: [$r(T) = C/(1+e^{k1+k2t'})$], where C= maximum rate of development at T_{opt}, k1 and k2 are constant and t'= T_{opt}- T for T≤ T_{opt} (Stinner *et al.*, 1974).

Linear equation: $[r(T) = \rho(T - T_b)]$, where ρ and T_b are estimated from standard linear regression procedures (Table in description Fig. 3.3).

Exponential-Tb model: [$r(T) = e^{\rho(T_T\ Tb)}$] where ρ and Tb are respectively the slope and intercept.

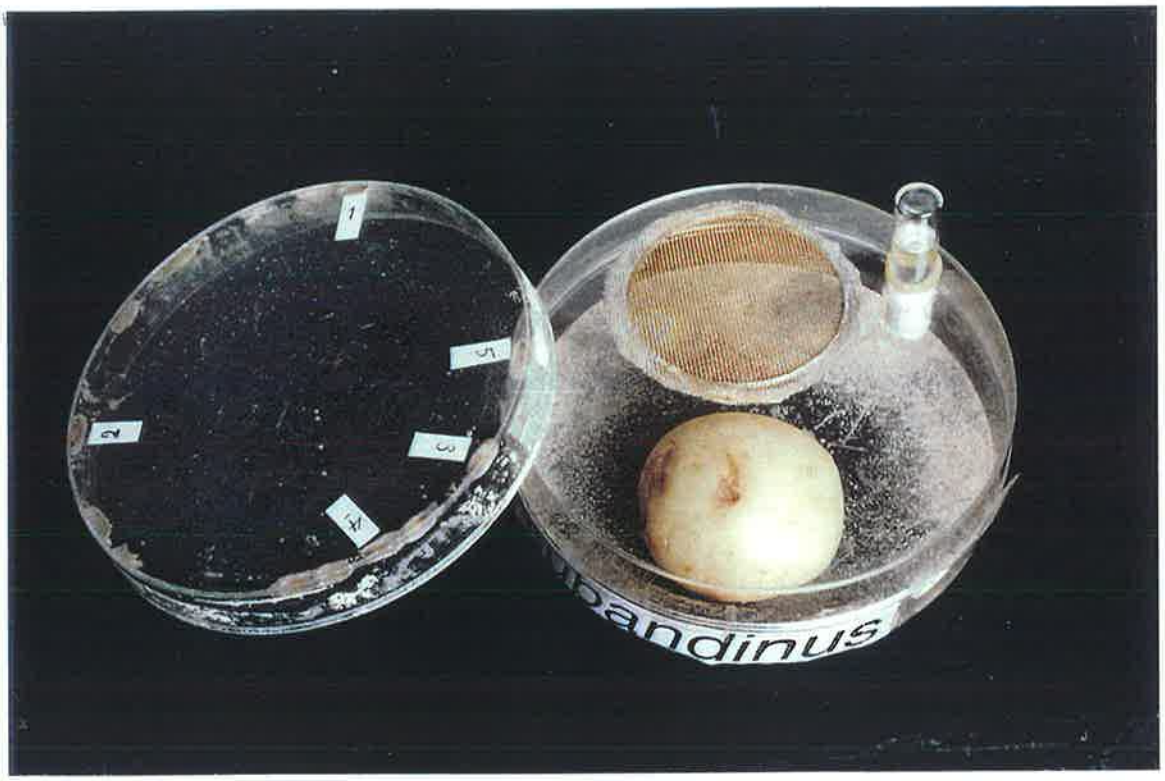


Figure 3.1: The insect rearing cage. The numbers on self-adhesive labels on the bottom of the cage show the positions of individual prepupae.

Adult longevity

Adults emerging from the development study were used to estimate adult longevity at the respective temperatures (24hr light). Male and female wasps were collected during each 12hr inspection and placed individually in glass vials, 30ml for *O. lepidus* and 20ml for *A. subandinus*. On the lid of each vial, wire mesh was provided to supply ventilation and an emulsion of honey and water for feeding. Vials were stored on their sides in petri dishes measuring 15cm × 2cm in diameter. The survival of the parasitoids was recorded at each daily inspection and a fresh drop of honey emulsion placed on the wire screen.

In an additional experiment, the longevity of male and female *A. subandinus* and *O. lepidus* that had been reared from eggs to adults in the insectary at 24°C was studied at five constant temperatures. Parasitoids (<12hr-old) were collected with an aspirator, placed individually into vials as described above, and held at 15, 20, 35, 30 and 35°C (24hr light). The aim of this part of the experiment was to study the longevity of adult wasps when transferred to different temperatures after emerging in the culture room.

Differences between the longevity of parasitoids reared in the incubators and reared in the culture room were analysed with ANOVA using Genstat 5 (Lane and Payne, 1996).

PTM development

The developmental times of larvae, prepupae and pupae of PTM were determined by rearing both PTM larvae held as controls and those which had not been parasitised among larvae exposed to parasitoids. To determine the developmental times of PTM eggs, 20-25 eggs newly laid on a tissue paper (Fig. 2.1.A) were placed in 30ml glass vials and transferred to the incubators. Observations were made twice daily at 12hr intervals to record hatching of eggs.

This test was repeated three times and the mean time of egg development was estimated for each temperature.

3.3 Results

Development

A. subandinus

The developmental rate of the combined egg and larval stages shows a curve smoothly increasing from low to high temperature whereas the rate of development decreased sharply at 35°C for prepupal and pupal stages of this parasitoid (Fig. 3.2). The minimum duration of development of *A. subandinus* was at 35°C. The mean time (±sem) required for development under constant temperatures ranged between 14.7 (±0.5) and 36.9 (±1) days, at 35 and 15°C respectively (Appendix 3.1). Therefore, it seems that the development time of the egg-larva of this parasitoid is less sensitive to high temperatures.

The data indicate a relative mortality of egg-larva varying from 22.2% at 30°C to 25.9% at 15°C. There was no mortality at the prepupal stage of *A. subandinus* but 5% and 15% of pupae of this parasitoid died at 15°C and 35°C respectively (Fig. 3.4.A).

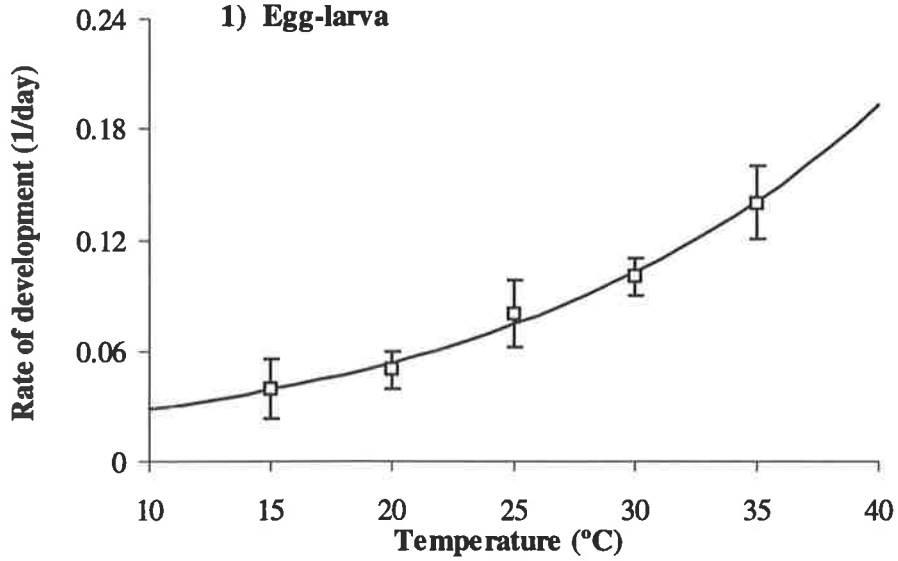
O. lepidus

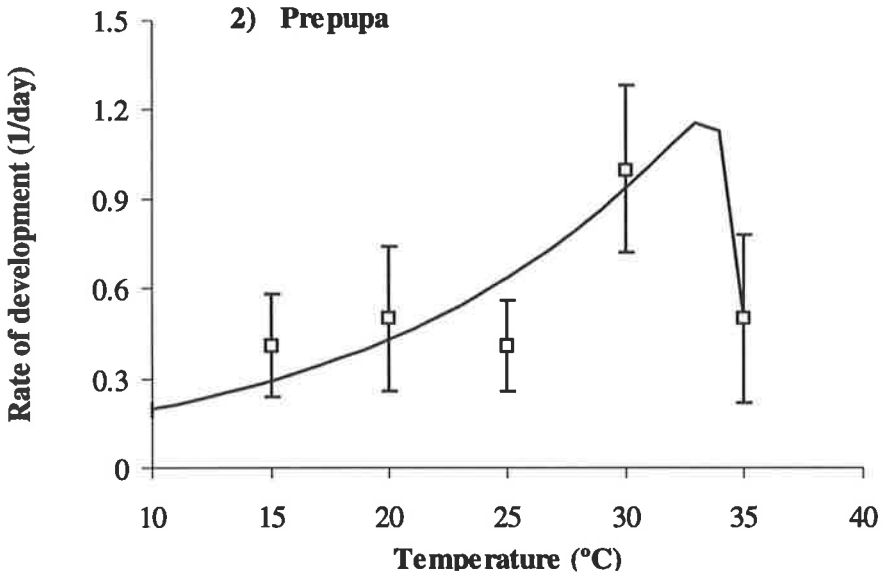
The rate of development of *O. lepidus* increased from 15°C to 35°C for all stages. The mean (±sem) developmental time from egg to adult emergence varied from 18.25 (±1.4) days at 30°C to 37.7 (±1.2) days at 15°C (Appendix 3.2). The adjusted coefficient of determination for the relationship between temperature and developmental rate of the egg-prepupa of this parasitoid was 0.97 (Fig. 3.3). In both species males were observed to emerge at least half a day (at 35°C) to 2 days (at 15°C) before females. The egg-larval and pupal stages of males

Figure 3.2: Relationship between temperature and developmental rate (1/days) for *A. subandinus* at 5 constant temperatures (15, 20, 25, 30 and 35°C). Curves were fitted with PMDS (Logan and Weber, 1991). Error bars represent sd. Parameters of curves as follows:

			Parameter							
Stage	Function	ACD*	ψ	ρ	ϒ	ΔT	c	k1	k2	T_{opt}
Egg- larva	Exponential	0.98	0.03	0.06	-	-	-	-	-	-
Prepupa	Logan I	0.25	0.20	0.07	2	0.5	-	-	-	-
Pupa	Stinner	0.86	-	-	-	-	4.89	5.34	0.07	31.6

* Adjusted Coefficient of Determination


1) Egg-larva
Rate of development (1/day)
0.24
0.18
0.12
0.06
0
10
15
20
25
30
35
40
Temperature (°C)


2) Prepupa
Rate of development (1/day)
1.5
1.2
0.9
0.6
0.3
0
10
15
20
25
30
35
40
Temperature (°C)

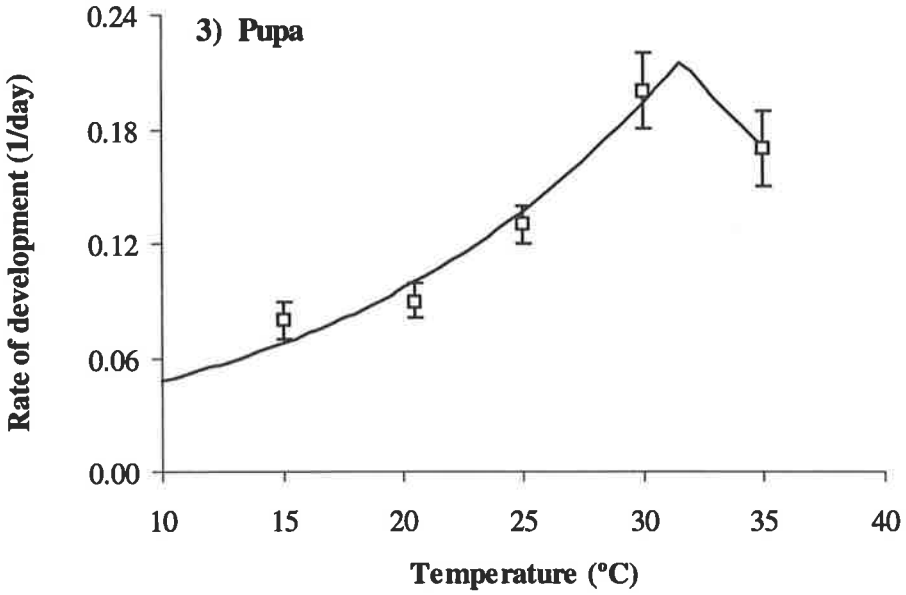
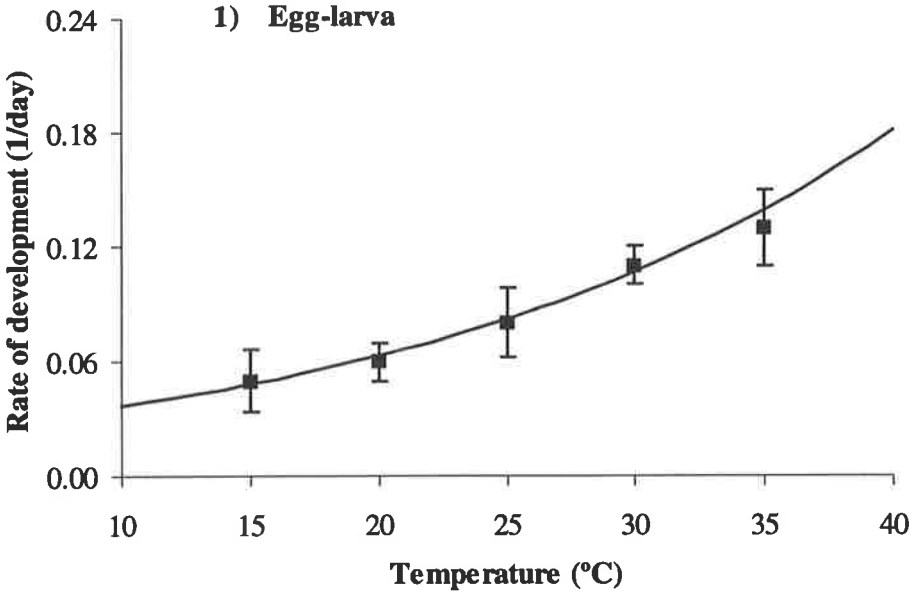
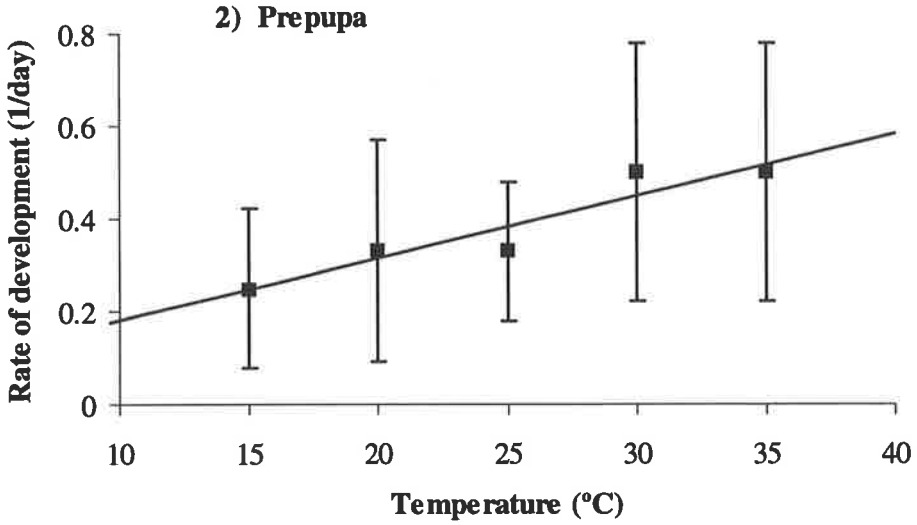

3) Pupa
Rate of development (1/day)
0.24
0.18
0.12
0.06
0.00
10
15
20
25
30
35
40
Temperature (°C)

Figure 3.3: Relationship between temperature and developmental rate (1/days) for *O. lepidus* at 5 constant temperatures (15, 20, 25, 30 and 35°C). Curves were fitted with PMDS (Logan and Weber, 1991). Error bars represent sd. Parameters of curves as follows:

			Parameter		
Stage	Function	ACD*	ψ	ρ	T_b
Egg- larva	Exponential	0.97	0.04	0.05	-
Prepupa	Linear	0.25	-	0.03	11.8
Pupa	Exponential	0.86	0.07	0.04	-

* Adjusted Coefficient of Determination


1) Egg-larva
Rate of development (1/day)
0.24
0.18
0.12
0.06
0.00
10
15
20
25
30
35
40
Temperature (°C)


2) Prepupa
Rate of development (1/day)
0.8
0.6
0.4
0.2
0
10
15
20
25
30
35
40
Temperature (°C)

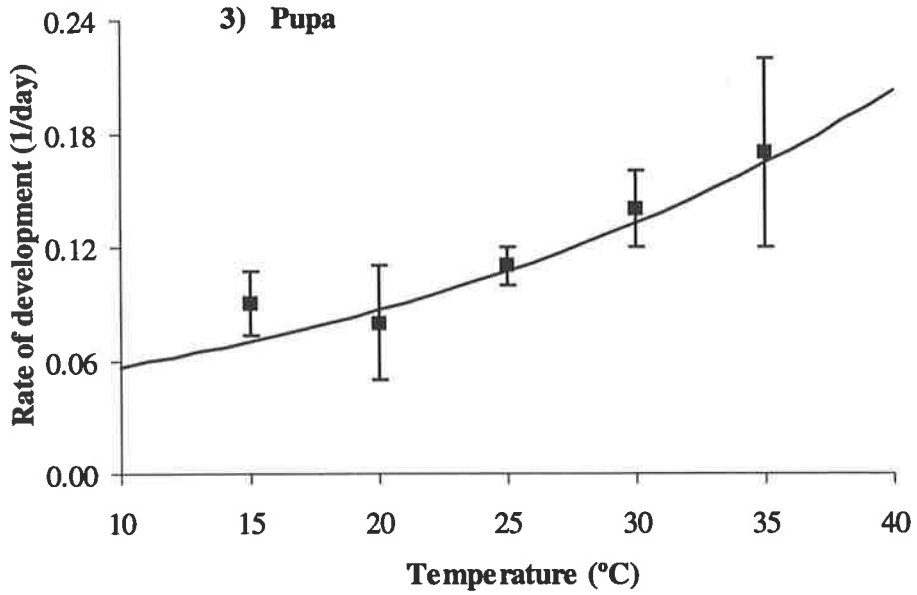

3) Pupa
Rate of development (1/day)
0.24
0.18
0.12
0.06
0.00
10
15
20
25
30
35
40
Temperature (°C)

Table 3.1: Effect of temperature on the longevity (days) of adult *A. subandinus*.

	Reared at given temperature						**Reared at 24 °C**					
Temp-erature	Median		Mean		n		Median		Mean		n	
(°C)	M*	F	M	F	M	F	M	F	M	F	M	F
15	16	18	16.8	17.2	18	12	18	26.5	17	26.5	14	10
20	13	16.5	14.7	15.7	18	7	16	17	16.1	17.4	14	9
25	13	15	13	14.1	16	10	15	18	13	16	11	8
30	9	9.5	7.78	8.5	14	9	9	7.5	7.25	8.73	11	8
35	4	6	4.67	5.9	12	8	2.75	3	2.7	3	10	7

* M - male, F - female

Table 3.2: Effect of temperature on the longevity (days) of adult *O. lepidus*.

	Reared at given temperatures						**Reared at 24 °C**					
Temp-erature	Median		Mean		n		Median		Mean		n	
(°C)	M*	F	M	F	M	F	M	F	M	F	M	F
15	9	13	12.1	15.5	17	10	9	14	13.3	16.5	17	13
20	16	18	16.7	19.4	17	13	14	15	14.7	16.9	17	13
25	15	17	14.7	17	17	13	13	16	14.4	16.2	17	14
30	8	10	7.8	9.1	17	16	5	8	5.8	7.4	17	13
35	2	4	2.8	3.9	11	7	3	4	3.1	4.2	15	13

* M - male, F - female

develop faster than females; a similar result has been reported for other braconid wasps (e.g. Nealis *et al.*, 1984). Considerable mortality of prepupae and pupae of *O. lepidus* was observed at 35°C (Fig. 3.4.B).

Adult longevity

The greatest longevity of *A. subandinus* was observed at 15°C (mean 16.8 days for males and 17.2 days for females) which was more than three times as great as at 35°C (4.7 days for males and 5.9 days for females) (Table 3.1).

The longevity of *O. lepidus* was inversely related to temperature except at 15°C when both sexes lived a shorter time than at 20°C. The maximum longevity of this wasp was at 20°C (mean 19.4 days for females and 16.7 days for males) and they had very short longevity at 35°C (mean 2.8 days for males and 3.9 days for females). Adult *O. lepidus* lived four times longer at 15°C than those at 35°C (Table 3.2).

In both *A. subandinus* and *O. lepidus,* data analysis indicated that there were no significant differences between the longevity of adults whose larvae were reared at five constant temperatures and those reared in a culture room at 24°C.

Males and females of the two species appeared to be smaller when reared at 35°C than those reared at 15°C, but their size was not measured. Markosyan (1993) also found that adult PTM reared at 35°C were smaller than those reared at 15°C. This suggests that when duration of feeding at high temperature is limited, larvae do not grow as large compared to those whose feeding continues for a longer time.

PTM development

The median rate of development of PTM increased with temperature in all immature stages. The data for PTM larvae show a good fit to the Exponential T_b model based on the largest

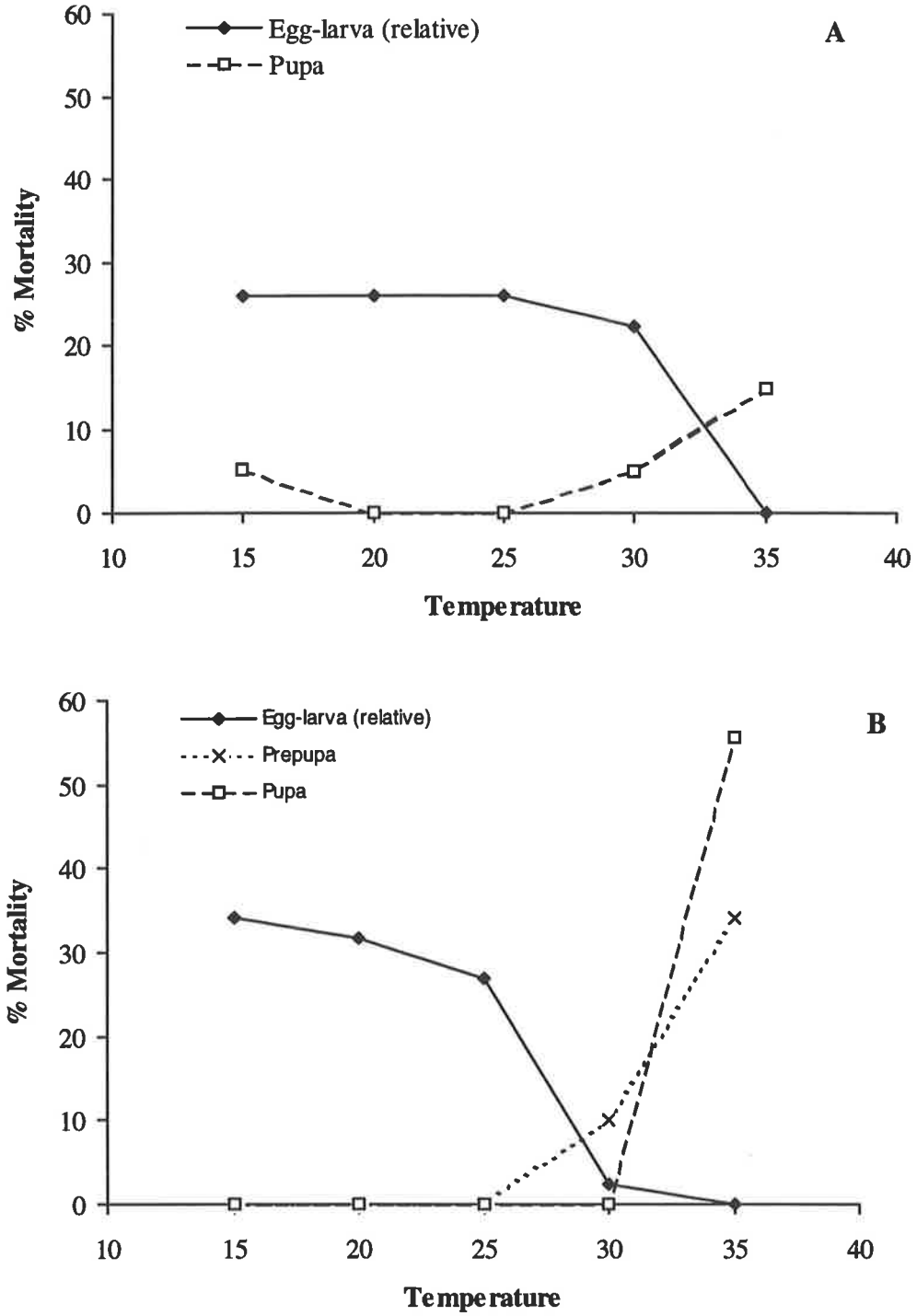


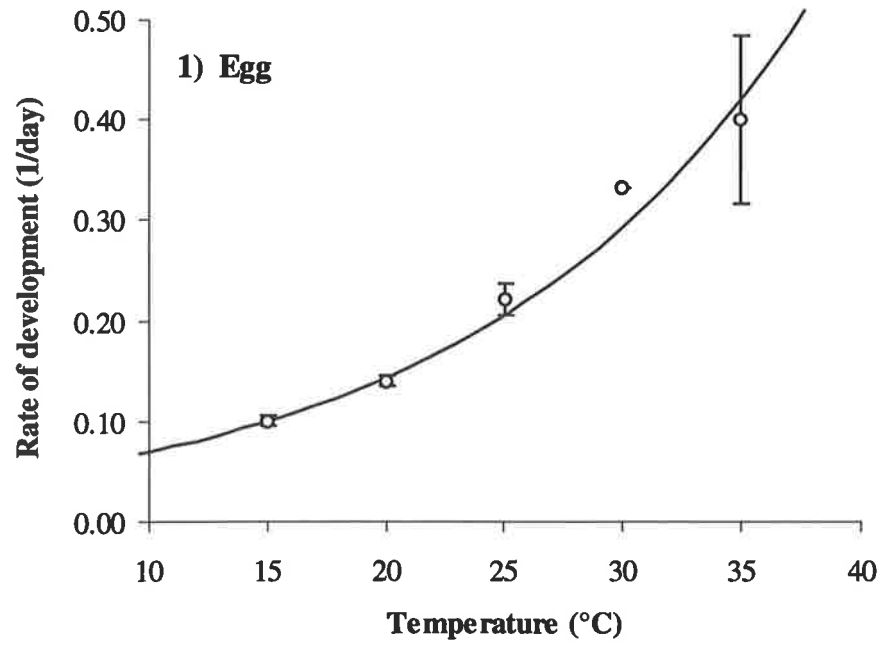
Figure 3.4: The percentage mortality for egg-larval, prepupal and pupal stages of parasitoids under constant temperatures (15, 20, 25, 30, and 35°C) obtained from experimental data.

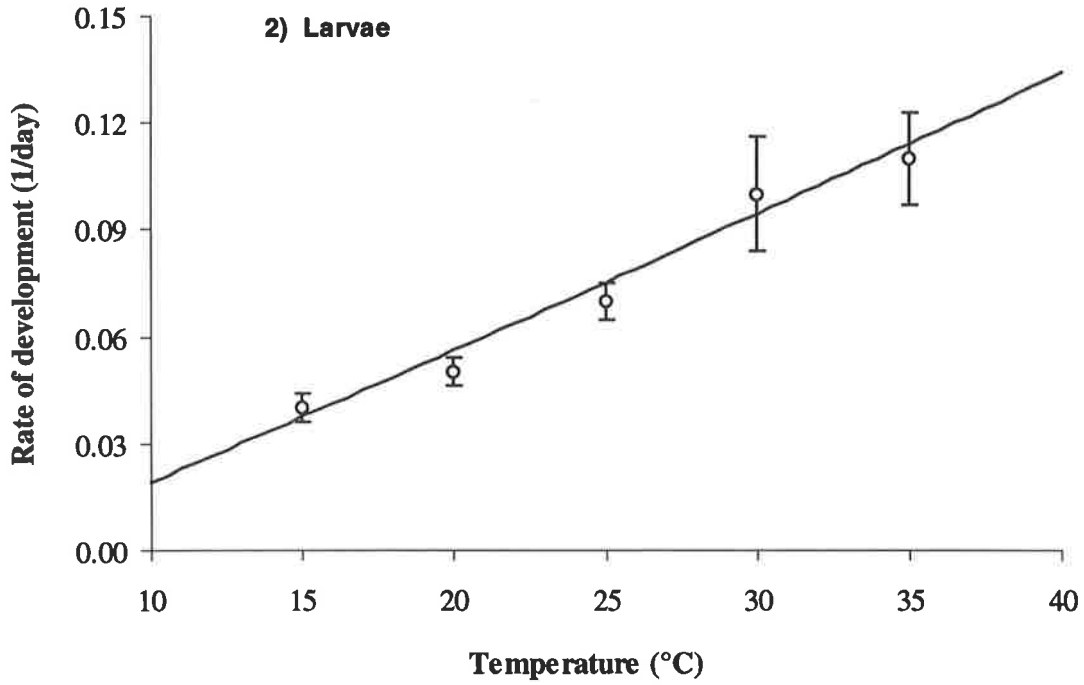
A) *A. subandinus* (no mortality in the prepupal stage), and **B)** *O. lepidus*.

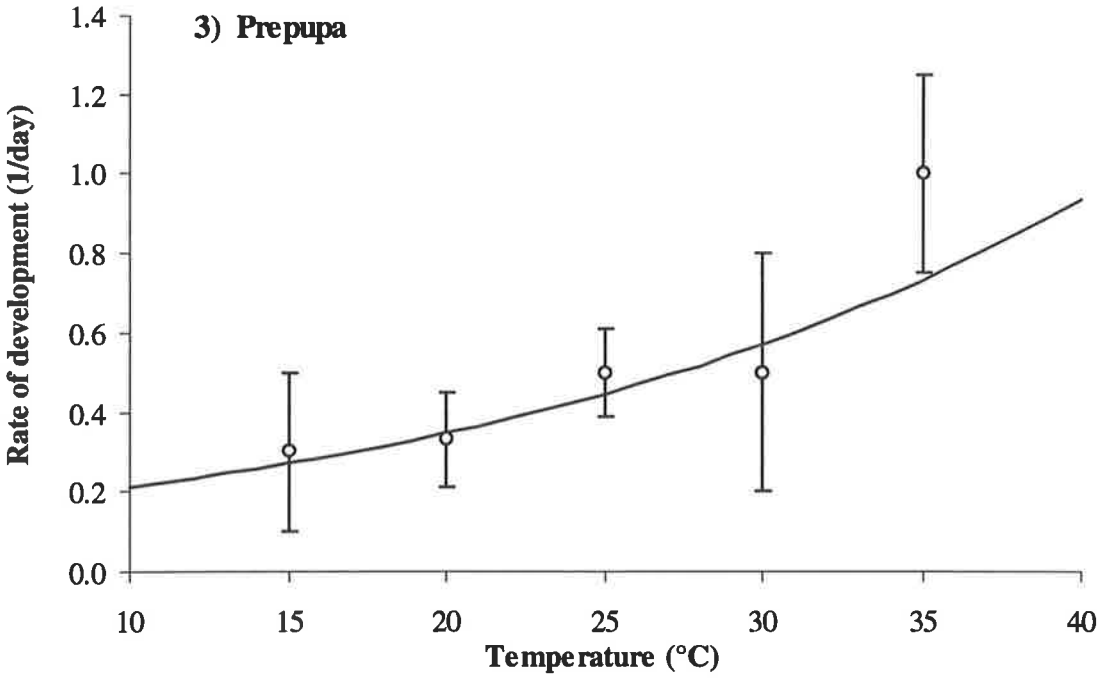
Figure 3.5: Relationship between temperature and developmental rates (1/days) for PTM at 5 constant temperatures (15, 20, 25, 30 and 35°C). Curves were fitted with PMDS (Logan, 1988). Error bars represent sd. Parameters of curves as follows:

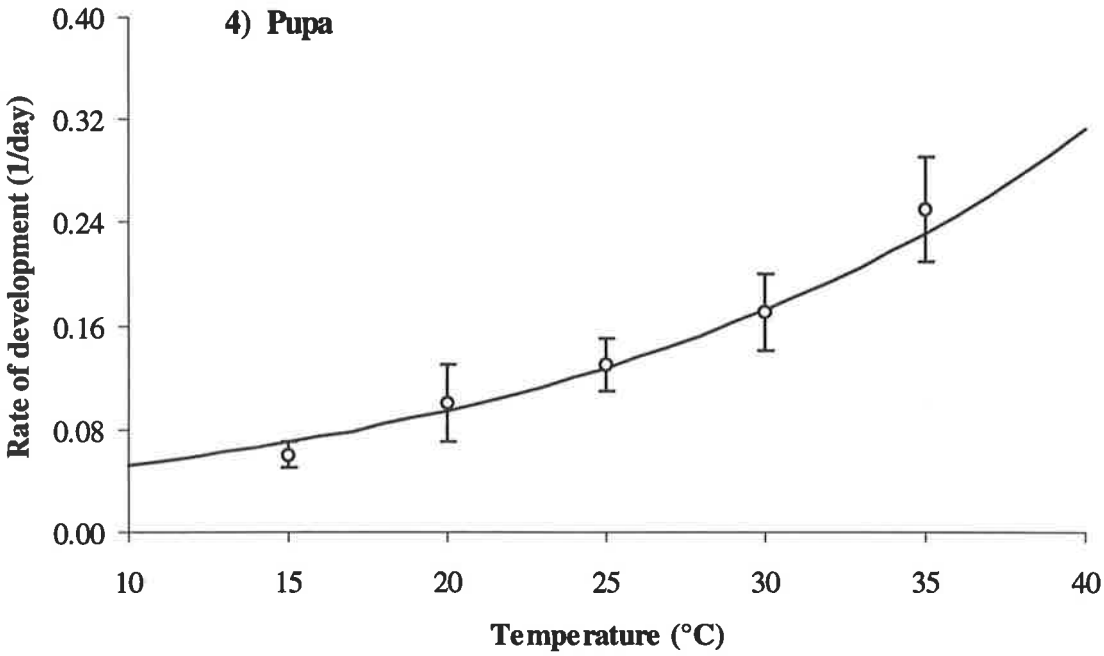
			Parameter		
Stage	Function	ACD*	ψ	ρ	T_b
Egg	Exponential	0.94	0.102	0.072	-
Larvae	Exponential-T_b	0.92	-	0.003	4.64
Prepupa	Exponential	0.75	0.027	0.056	-
Pupa	Exponential	0.98	0.070	0.061	-

* Adjusted Coefficient of Determination


1) Egg
Rate of development (1/day)
0.50
0.40
0.30
0.20
0.10
0.00
10
15
20
25
30
35
40
Temperature (°C)


2) Larvae
Rate of development (1/day)
0.15
0.12
0.09
0.06
0.03
0.00
10
15
20
25
30
35
40
Temperature (°C)


3) Prepupa
Rate of development (1/day)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
15
20
25
30
35
40
Temperature (°C)


4) Pupa
Rate of development (1/day)
0.40
0.32
0.24
0.16
0.08
0.00
10
15
20
25
30
35
40
Temperature (°C)

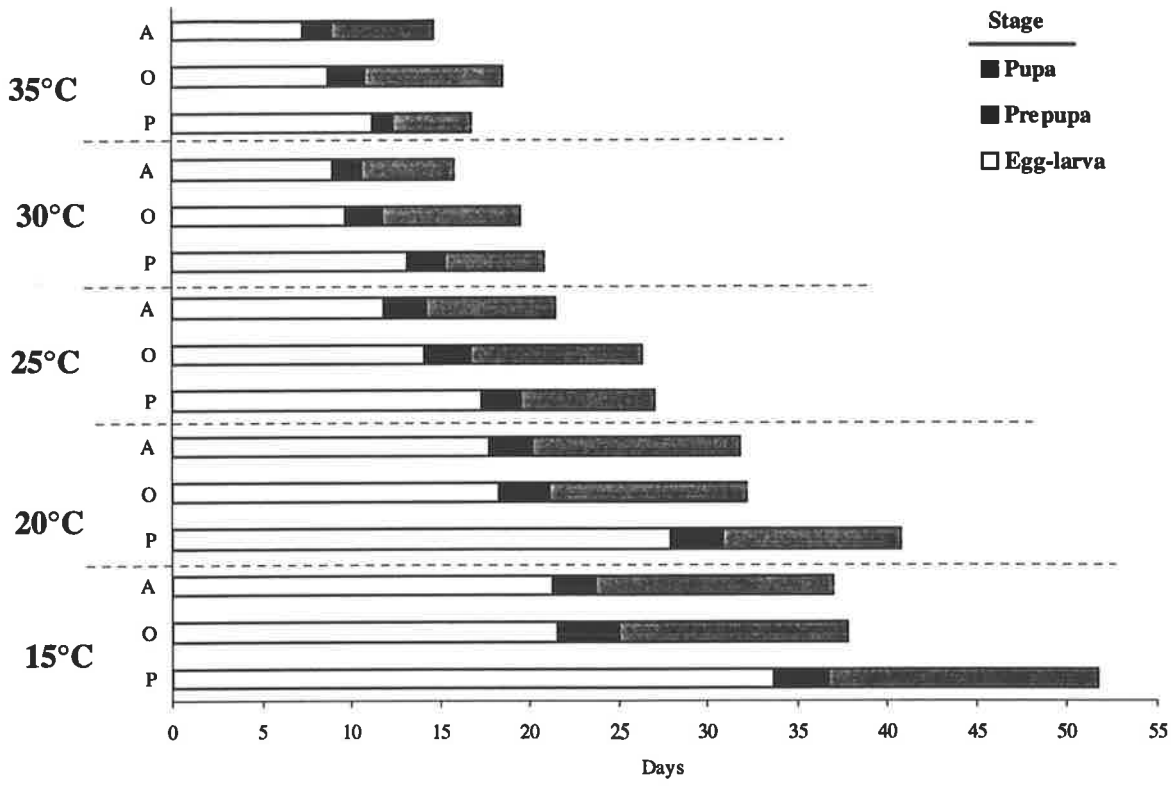


Figure 3.6: Development time (egg-adult) for *A. subandinus*, *O. lepidus* and their host PTM at five constant temperatures.

(A= *Apanteles subandinus*, O= *Orgilus lepidus* and P= PTM, potato tuber moth).

adjusted coefficient of determination and the Exponential model provided the best fit for egg, prepupae and pupae (Fig. 3.5). The rate, range and mean duration of development of larvae, prepupa and pupa PTM are presented in Appendix 3.3. Eggs of PTM of the same age hatched within one day at each temperature. Experimental data show a faster development at 35°C and slower at 15°C for PTM stages.

Comparison between the median growth rate for all stages of PTM and the two parasitoids indicates that they gave similar responses to changes of temperature (Fig. 3.6). However, the mean developmental time for one generation of PTM is longer than for *A. subandinus* or *O. lepidus* at all temperatures except *O. lepidus* 35°C (Appendixes 3.1-3.3).

3.4 Discussion

The results of this study indicate that completion of development of both parasitoid species is faster than PTM, except for *O. lepidus* at 35°C. The development time of *A. subandinus* between 15°C and 35°C is shorter than *O. lepidus*, and *A. subandinus* lives slightly longer at very low and high temperatures compared to *O. lepidus*. Development time of the two parasitoids and their host increased at lower temperatures. At low temperatures the difference between the duration of the parasitoid generations and that of PTM was much greater than at high temperatures. The duration of the egg-larval stage of the two parasitoids and their host was longer than the duration of their prepupa and pupae stages and the largest difference in their development time was at the egg-larva stage. The effects of temperature before pupation of the parasitoids may be indirectly due to the effects of temperature on the host. Development rate showed the same trends in the PTM and the parasitoids.

Parasitoids and PTM also appeared to reach a larger size when reared at lower temperatures. Markosyan (1993) reported an increasing in the weight of pupae with decline of temperature. Possibly, increasing size of these parasitoids in low temperature depend on size of PTM.

Cardona and Oatman (1975) found that *A. subandinus* did not develop at 35°C. However, the present study showed 40% mortality at the pupal stage at this temperature. Duration of development at other temperatures was slightly shorter in the American than in the Australian strain, except at 15°C where the American strain took longer to develop. This result confirmed that the effects of environmental elements on the development of insects commonly differ from location to location (Danks, 1992). Thirty years after the introduction of the two species into Australia, they may have adapted to the new environment which has a different climate, and host plants. Their host is also adapted to the changed conditions (Briese, 1986). The nutritional value of the host plants could also have differed between the Californian study and the work reported here. Horne (1990) found that *A. subandinus* was dominant in crops early, but was replaced by *O. lepidus* later. Perhaps the ability of *A. subandinus* to develop more rapidly at lower temperatures has influenced their seasonal abundance.

The difference in tolerance to high temperatures between the larval and pupal stages of the two parasitoids is noteworthy. Prepupal and pupal development of *A. subandinus* was retarded at 35°C, while the pupae of both parasitoids suffered some mortality at this temperature (Figures 3.2-3.4). In the field, larvae feeding in foliage would often experience temperatures of 35°C or more during summer. Pupae in the soil are buffered from extreme variations in temperature, especially those that are in soil shaded by host plants. Thus pupae probably do not experience the same temperature extremes and as such have not adapted to develop at the same high temperatures that larvae tolerate.

Females of both parasitoids lived longer than males of the same species. The longevity of *O. lepidus* is less than *A. subandinus* at low and high temperatures (15°C and 35°C) and greater than that of *A. subandinus* at intermediate temperature of 20-30°C. The short life span of *O. lepidus* at 35°C indicates that it does not survive well at high temperature and may not live long enough to have an effect on its host in terms of rate of parasitism. The longevity of adult

parasitoids was mostly unaffected by rearing temperatures in the range 15 - 35°C. However, longevity of adults at 24°C was a few days shorter than those reared at the other 5 temperatures.

The developmental rate of PTM at five constant temperatures was similar to results that Horne and Horne (1991) and Markosyan (1993) reported for this insect. The life-cycle of PTM was completed in an average of 27 days at 25°C in contrast with results reported by Salas and Quiroga (1985) who estimated 34.3 days at 24°C. Here too, nutritional differences could affect the rate of host develoments.

Coexistence of parasitoid populations of different species requires that some differences exist in niches between the species (Gause, 1934). Possibly, different tolerance of extreme temperatures is one of the factors influencing niche segregation of the two parasitoids at different times of the crop growing season.

In conclusion, the results from this study predict that *A. subandinus* and *O. lepidus* should be well-adapted to local environment over a range of temperatures from 15°C to 35°C. *A. subandinus* is predicted to be better adapted to very low and high temperatures than *O. lepidus*. *O. lepidus* was very susceptible to high temperature and at 35°C most of the parasitoid did not complete development, dying mostly in the pupa stage. Those that survived to became adults lived only 2 to 3 days. *A. subandinus* was more tolerant of high temperatures than *O. lepidus* which showed high mortality at 35°C. Therefore, at high temperatures *A. subandinus* had an advantage over its host, while growth of *O. lepidus* was retarded compared with that of PTM. These data suggest that for biological control of PTM, an inoculative release of *A. subandinus* early in the growing season and release of *O. lepidus* later when weather is warmer could increase parasitism in the field.

Chapter Four

Reproductive capacities of *A. subandinus* and *O. lepidus*

"Reproduction of a parasitoid is a function of the individual and it is depend to host-parasitoid interactions and parasitoid egg-laying capacity"

P. DeBach and H. S. Smith, 1941

4.1 Introduction

The impact of a parasitoid population on a population of its host depends upon several interrelated factors. Among these, the ability of the parasitoid population to increase is of special importance. [IC1]In nature the action of biotic factors and the complex interrelations among them are important in the reproduction of an insect (DeBach and Smith, 1941). Variation in fecundity is an important influence on population density (Dempster, 1975). Variations in temperature, host density, and parasite density can also affect parasitoid reproduction. However, the fecundity of a species largely depends on the capacity of the ovaries to produce a given number of eggs (Engelmann, 1984).

The biology of *A. subandinus* and *O. lepidus* has been studied on PTM infested potato tubers by Cardona and Oatman (1975), and Oatman *et al.* (1969). However, there is no information about the reproduction of the two parasitoid species when host larvae are distributed among host plant leaves in conditions similar to those found in the field, or when host density or temperature are varied.

Understanding the reproductive capacities of *A. subandinus* and *O. lepidus* will be useful as one of the important factors in decision making for pest management programs. For effective augmentative control of a PTM population, it would be necessary to release sufficient numbers of parasitoids in relation to the number of PTM estimated to be present in an infested field (Callan, 1974).

In this chapter, the reproductive capacities of the parasitoids *A. subandinus* and *O. lepidus* and their sex ratios are reported under laboratory and field conditions in six experiments.

4.1.1 Aims

The hypotheses for this study were that the reproductive rates of *A. subandinus* and *O. lepidus* vary with host density, exposure period, host plant tissues and temperature. To address these hypotheses, the aims of these experiments were:

1) to estimate the reproductive activity of the Australian strains of *A. subandinus* and *O. lepidus* on potatoes with different host densities;

2) to compare the egg-laying capacity of each species during different periods of exposure;

3) to measure the parasitoids' egg-laying ability when exposed to either infested potato tubers or foliage;

4) to investigate the effects of variations in temperature on the reproductive ability of the two parasitoids;

5) to compare the sex ratios of wild populations of parasitoids with those reared in the laboratory.

4.2 The reproduction of the two parasitoid species with different host densities, host plants and duration of exposure

Four series of experiments were conducted in this section. The first focused on the effect of host density on egg-laying during the lifespan of a female of each species. In the second, the egg-laying activity of females was measured when each individual female was offered a choice of different host densities. The third determined whether egg-laying was affected by the potato tissues (leaf or tuber) on which the host was feeding. In the fourth, the egg-laying capacity of females was related to exposure time.

4.2.1 Materials and methods

In laboratory tests, the reproductive biology of the two parasitoid species was investigated in a controlled-environment room at 24°C with 60 - 70% RH and a light regime of 14L : 10D using cool-white fluorescent lights.

The gravid female wasps used in this study were taken from a stock culture in the insectary (Section 2.2). Since numerous investigations (e.g. Engelmann, 1984) have shown that better egg production by parasitoids depends on having well-fed hosts, extra potato tubers provided a surplus of food for PTM larvae during rearing.

a) Four densities of hosts exposed separately to parasitoids

Newly emerged larvae of PTM were uniformly distributed among the leaves of each potted potato plant of equal size (30-40cm) at four host densities (20, 40, 80 and 160 larvae per plant). The infested plants were transferred to oviposition cages (Fig. 4.1.A). One 1-day-old gravid female parasitoid was introduced to each cage. The wasps were provided with drops of honey on the perspex at the top of the cages and cotton wicks saturated with water. After 24hr, the plants were exchanged with fresh infested plants with same number of PTM. This process was repeated every day until the parasitoids died of old age, when they were dissected to find the number of eggs remaining in the ovaries.

The PTM larvae on previously exposed pots were transferred to clear plastic boxes (1 litre) with fresh potato tubers for larval feeding. Fine sand was provided for the pupation of larvae (Fig. 2.1.B). The number of wasps, their sex, and the number of moths emerging from the culture were recorded to estimate the parasitoids' daily reproduction and the actual density of hosts available for parasitisation.

The net reproductive rate (R_0) was estimated for the two parasitoids at different host densities using an equation described by Southwood (1978): ($R_0 = \sum l_x m_x$), where x is age of females in days, l_x refers the proportion of females alive at age x and m_x is the number of living females born per female in each age interval and it is estimated by: $m_x = N_x / 2$, where N_x is the total natality per female of age x.

b) Simultaneous exposure of four host densities to parasitoids

In this series of tests each female parasitoid was exposed for 3 days to four nominal densities of PTM (i.e. 20, 40, 80 and 160 larvae per potted potato) in a cage ($55 \times 50 \times 40$cm) (Fig. 4.1.B). After this period the infested foliage was placed in separate rearing boxes for each density. The numbers of emerged insects and hosts parasitised were recorded for each density. The data obtained from this experiment were analysed with simple regression using Genstat 5 (Lain and Payne, 1996).

c) Host plant tissue

An experiment was set up to estimate the rate of egg-laying by the two parasitoid species on tubers as compared to infested potato foliage. In this experiment, potato tubers of similar size (see parasitisation unit in Section 2.2) and potted potato plants were each infested with 50 newly emerged PTM larvae. After 24hr infested tubers and foliage were placed in separate cages and exposed to a newly emerged gravid female wasp (each species individually) for 24hr.

This experiment was repeated 9 times for *A. subandinus* and 10 times for *O. lepidus*. Differences in the number of progeny produced per female per day on each plant tissue by the two parasitoids were compared by the Kruskal-Wallis method using Genstat 5 (Lane and

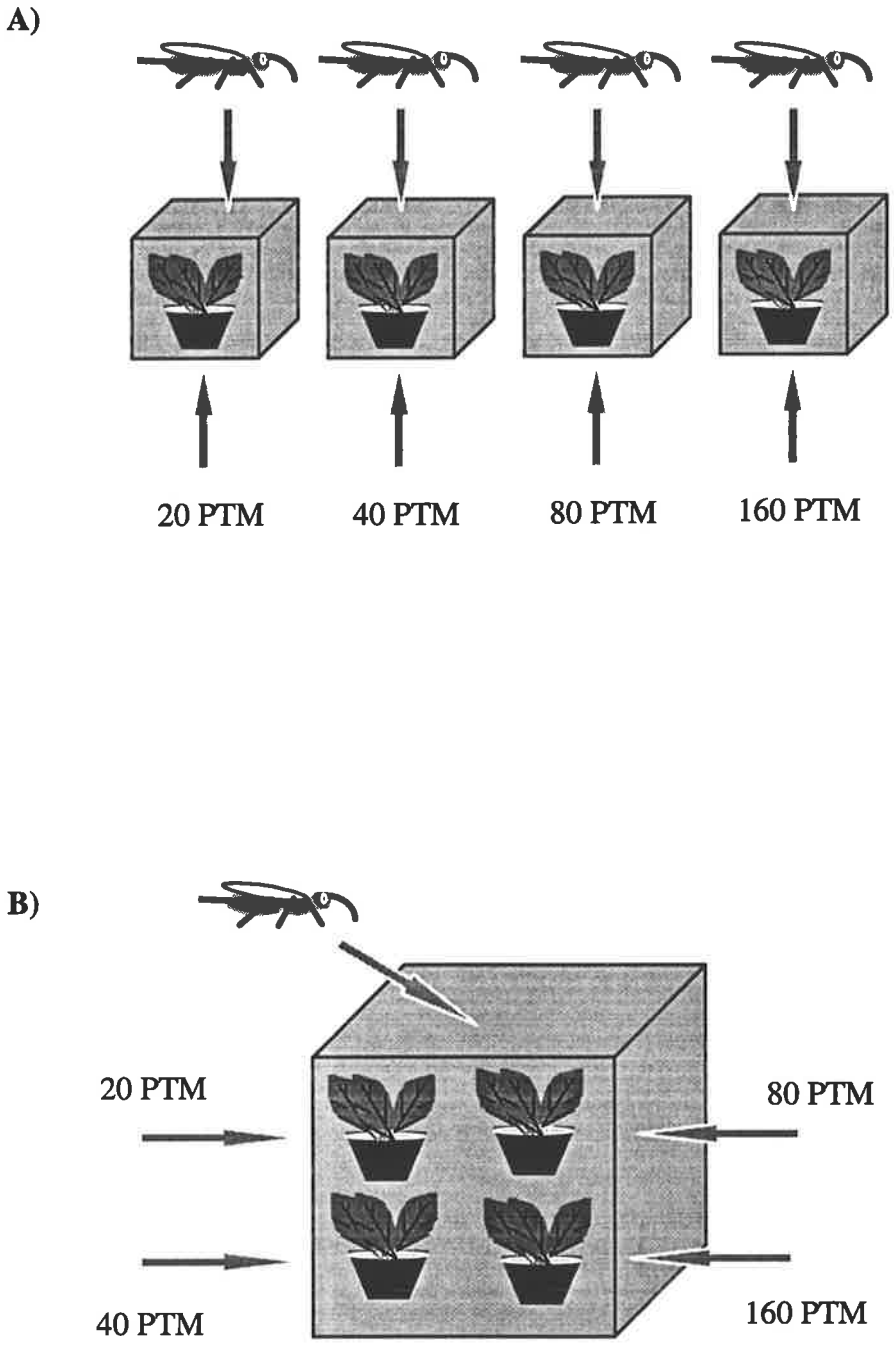


Figure 4.1: Schematic representation of cage array used to release parasitoids.

A) Four host densities introduced to individual parasitoid separately, **B)** four host densities introduced to individual parasitoid simultaneously.

Payne, 1996).

d) Time-dependent egg-laying

In this series of tests, exposure time was varied from 4hr to 24hr for a constant number of hosts. The aim was to confirm that by increasing exposure time the rate of parasitism by the parasitoids would increase and also to look for a diminishing rate of oviposition over time.

Infested potato tubers, each with 50 newly emerged PTM larvae, were exposed to individual female wasps for different exposure times (4hr, 8hr, 12hr and 24hr). The exposed larvae were reared until eclosion and the number of adults was recorded. The obtained data from oviposition by each wasp species was analysed with regression analysis using Genstat 5 (Lane and Payne, 1996).

4.2.2 Results

a) Reproduction at different host densities

Females of both species commenced egg-laying during the first day after emergence. Egg-laying by females fluctuated daily and the rate of oviposition decreased with age. More hosts were parasitised by both species as the number of hosts per plant increased (Fig. 4.2).

The establishment of PTM larvae on plants ranged between 52.7% at the highest density (160 larvae/plant) to 64.4% at lowest density (20 larvae/plant). The failure of substantial numbers of host larvae to establish on leaves and tubers was observed in all experiments, including those described in other chapters. It is likely many newly emerged larvae dispersed from the potato plants. Varela and Bernays (1988) found that the newly hatched first instar larvae had rapid movement. They crawl quickly and fall from the surfaces

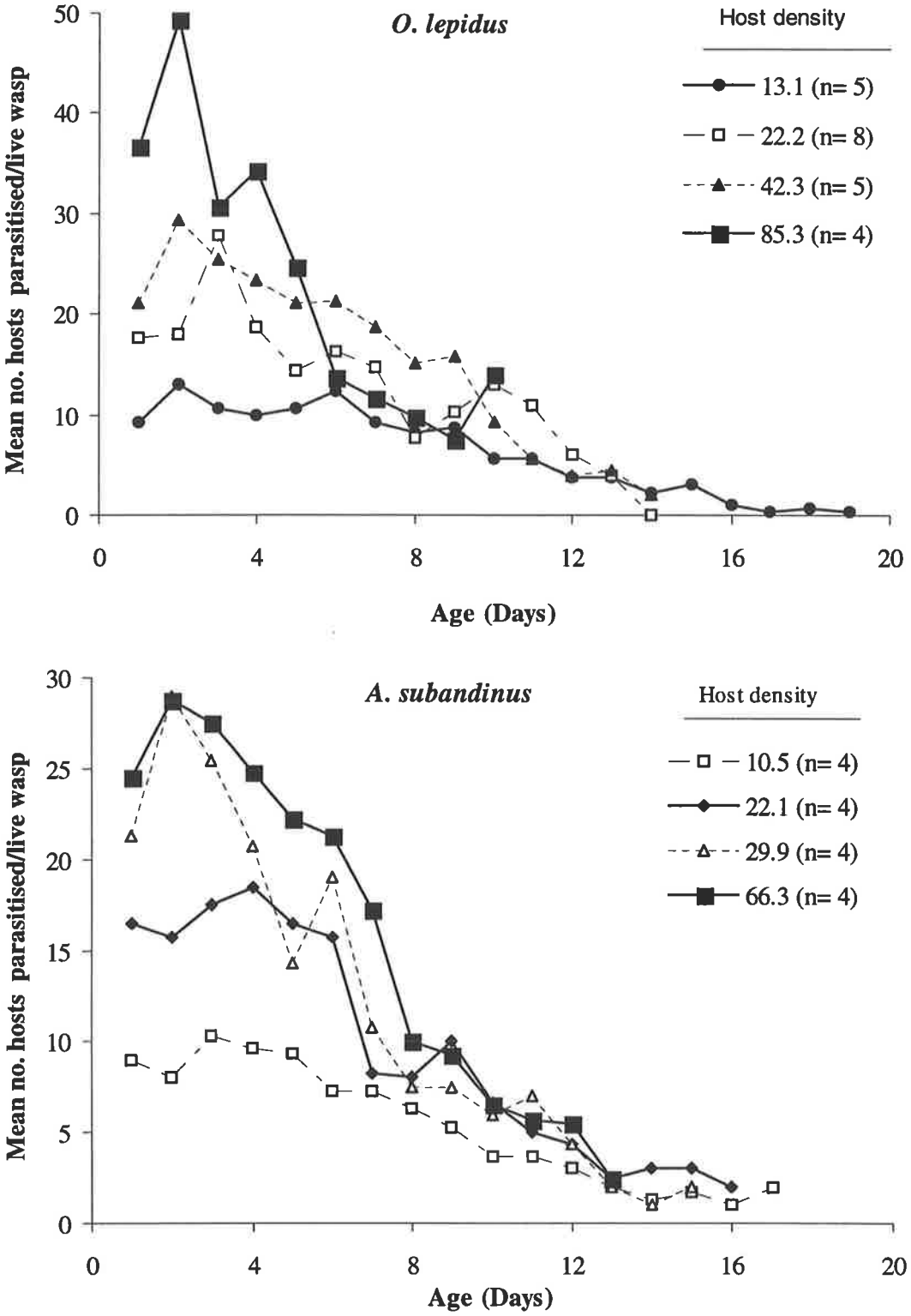


Figure 4.2: Mean daily reproduction (egg/female/day) by *A. subandinus* and *O. lepidus* at different host densities in the laboratory.

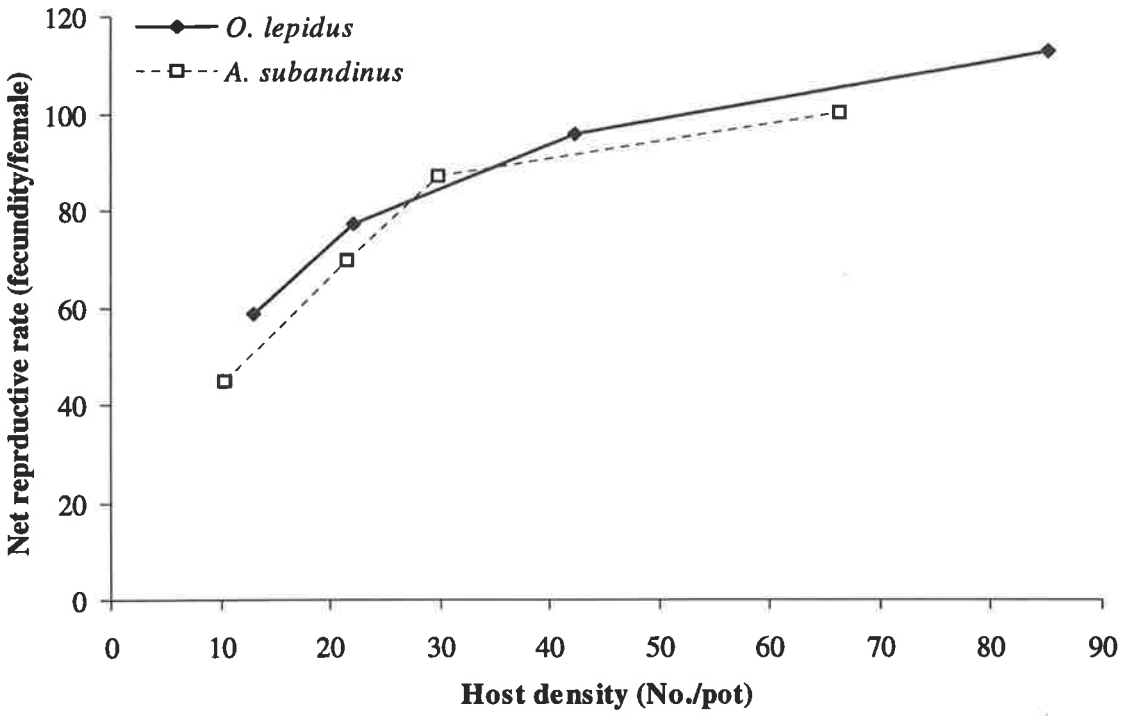


Figure 4.3: The net reproductive rate (R_0) of the two parasitoids when host density increased.

Table 4.1: Longevity of female *O. lepidus* at different host densities.

Host density	Minimum (days)	Maximum (days)	Mean	sd.	n
13.1	8	18	14.0	3.8	5
22.2	8	13	11.0	2.1	7
42.3	6	14	10.6	3.0	5
85.3	8	12	9.8	1.7	4

Table 4.2: Longevity of female *A. subandinus* at different host densities.

Host density	Minimum (days)	Maximum (days)	Mean	sd.	n
13.1	15	17	15.8	1.0	4
22.2	11	16	13.0	2.2	4
42.3	9	15	12.3	2.5	4
85.3	10	13	11.8	1.5	4

of host plants. These fallen larvae do not subsequently establish themselves on the plants. There is no information about their establishment in the field. Possibly, the percentage of missing larvae in the field is less than in the laboratory.

Net reproductive rate gradually increased when host density increased (Fig. 4.3). There was an inverse relationship between parasitoid longevity and host density particularly for *O. lepidus*. Longevity of the two species was shorter when they were introduced to high host density than low density (Tables 4.1 and 4.2). This is possibly due to female wasps expending energy. When wasps oviposited in more hosts per day at a high host density, they lived for a shorter period than when they oviposited in fewer of hosts.

b) Simultaneous exposure of four densities of the host

The numbers of hosts parasitised by each female *O. lepidus* and *A. subandinus* increased in this test from lower to higher host densities (Fig. 4.4). Both species oviposited in more hosts in high host density during the three days exposure. However, the coefficient of determination (R^2) for *O. lepidus* (0.83) was twice that of *A. subandinus* (0.41).

The results from these experiments also indicated a highly significant difference ($P< 0.05$) between the number of eggs laid by *A. subandinus* and *O. lepidus* in all replicates in the same conditions of densities of PTM.

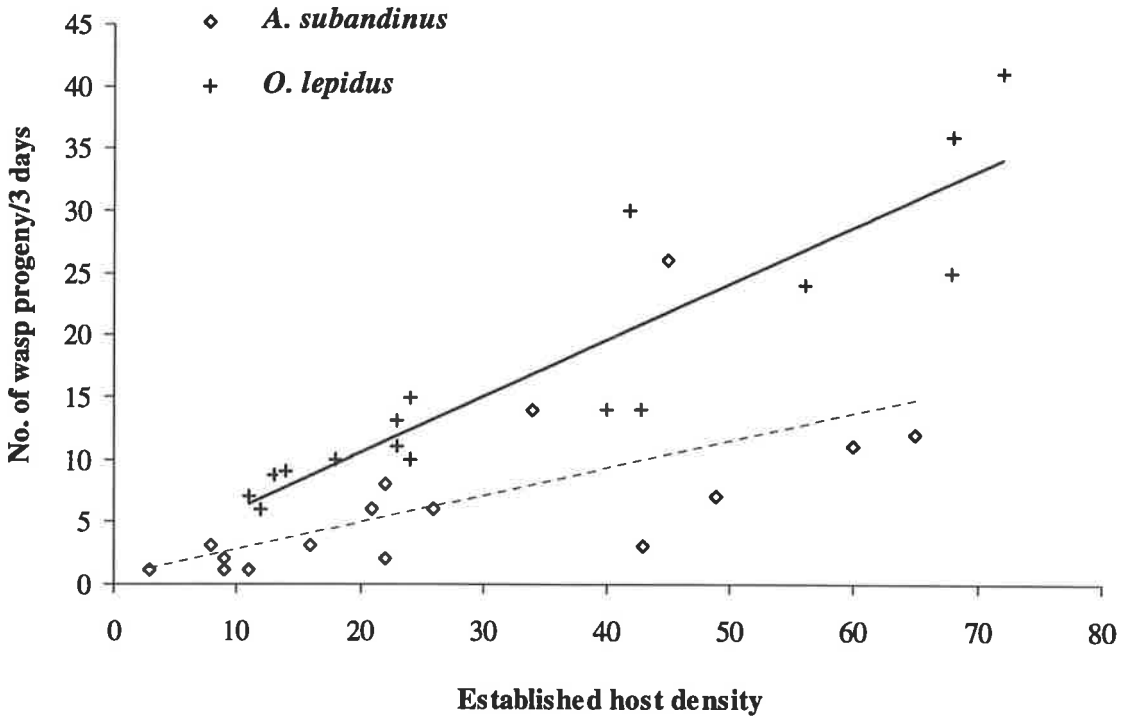


Figure 4.4: Comparison of the mean number of progeny of *A. subandinus* and *O. lepidus* when 4 infested pots with different host densities were exposed simultaneously to a female of each species individually.

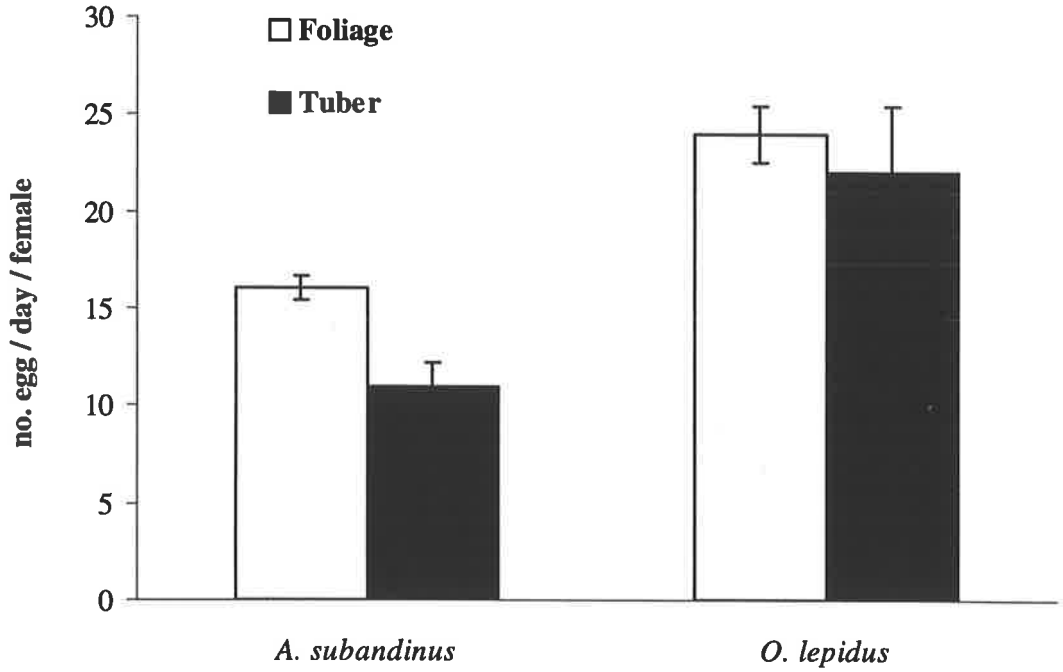
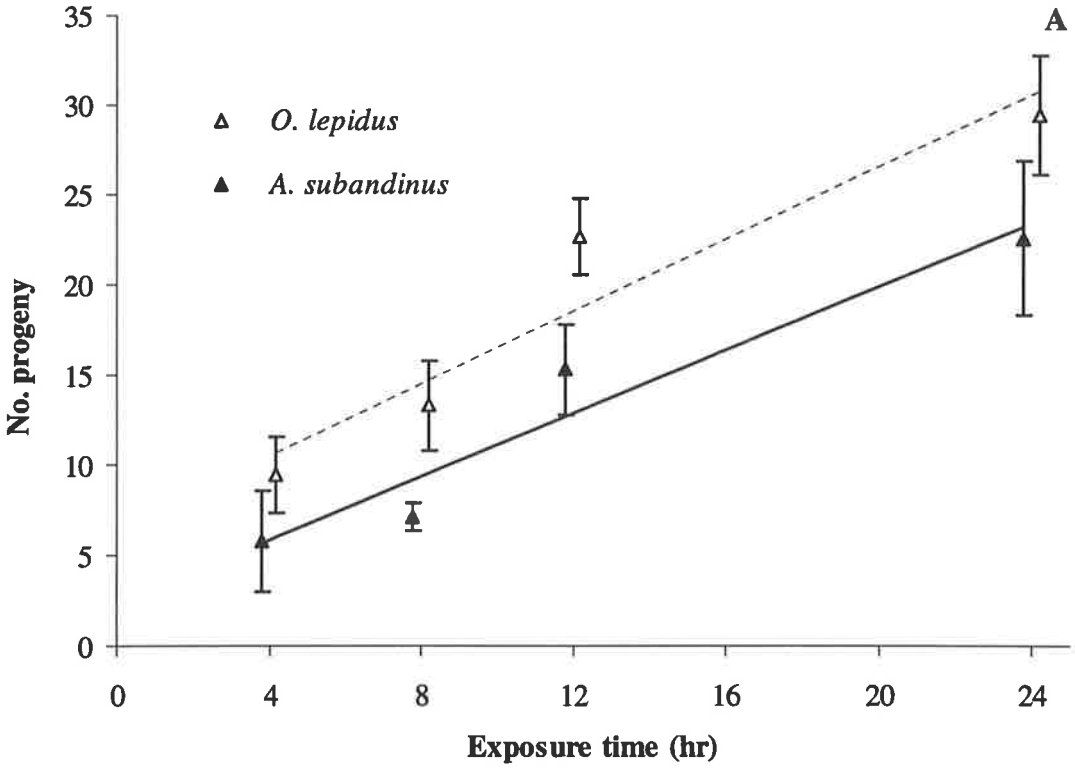


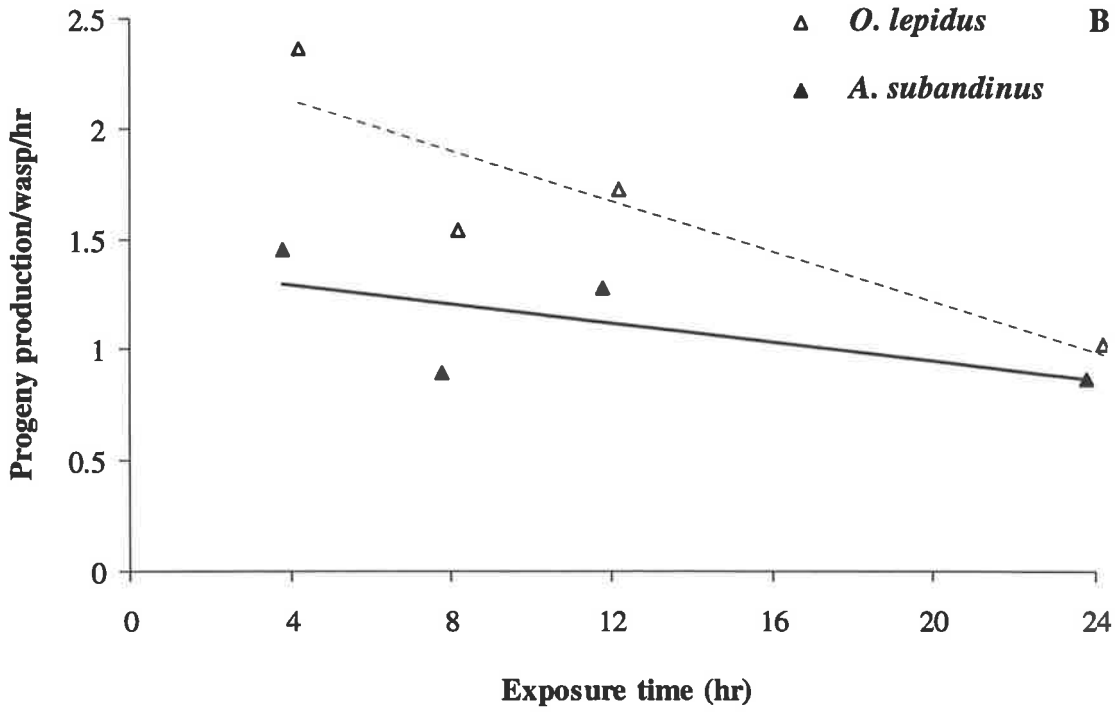
Figure 4.5: Eggs laid by *A. subandinus* and *O. lepidus* when infested foliage or infested tubers of potatoes with PTM (mean established =27.9 ± 3.4) were exposed to one female wasp for one day. Error bars show standard errors of means, n= 5 for each species, *P*= 0.008 for *A. subandinus* and *P*= 0.629 for *O. lepidus* (t-tests, Genstat).

Figure 4.6: **A)** Number progeny of *A. subandinus* and *O. lepidus* at different exposure times at a constant density of hosts in the insectary. Solid and open symbols show the mean number of progeny per female and solid and broken lines depict best fit linear regression model.

B) Rate of progeny production of *A. subandinus* and *O. lepidus* at different exposure times at a constant density of hosts in the insectary.

Regression of progeny production for female *A. subandinus* per hour was Y= -0.125X + 1.06, R^2= 0.18, and for female *O. lepidus* per hour was Y= -0.052X + 2.074, R^2= 0.78.


A
O. lepidus
A. subandinus
No. progeny
Exposure time (hr)
0
5
10
15
20
25
30
35
0
4
8
12
16
20
24


B
O. lepidus
A. subandinus
Progeny production/wasp/hr
Exposure time (hr)
0
0.5
1
1.5
2
2.5
0
4
8
12
16
20
24

c) Host plant selection

The mean number (±sd) of PTM established on foliage in all trials (19 replications) was 28.9 (±3.6) larvae per plant and on potato tubers was 26.9 (± 2.9) larvae per tuber. There was a significant difference (P= 0.008) between the numbers of hosts parasitised by *A. subandinus* when PTM larvae mined on tubers compared to foliage, whereas no difference was observed in *O. lepidus*. However, the rate of egg-laying by *O. lepidus* females was higher than females of *A. subandinus* in both infested potato tubers and foliage (Fig. 4.5).

d) Time dependent oviposition

The number of hosts parasitised by females of both species increased with increasing exposure time (Fig. 4.6.A). Individual females of both species laid twice as many eggs at 24hr exposure time than at 4hr ($p< 0.05$). The egg-laying of *O. lepidus* was correlated positively with the observed number of eggs produced by females of this species. The rate of oviposition per hour per wasp decreased with increasing host density (Fig. 4.6.B).

4.2.3 Discussion

For both species, the rate of egg-laying was influenced by PTM density. Both species had a different daily egg-laying capacity with changing host densities. These data show that more eggs were laid by *O. lepidus* when the number of hosts per plant was higher than with a low host density and similar results were found for *A. subandinus*. Figure 4.3 shows that with an increase in host density, the number of hosts attacked by *O. lepidus* and *A. subandinus* increased. The number of hosts parasitised on infested tubers was lower than on infested foliage. PTM larvae grow in potato leaves, stems and tubers, and gradually penetrate deeply into foliage and tubers. *O. lepidus* has greater success than *A. subandinus* with ovipositing in PTM larvae that have mined deeply into tubers. This difference appears to be due to the

length of the ovipositor, which is 7 times longer in *O. lepidus* (4.5mm) than in *A. subandinus* (0.63mm).

4.3 Counting eggs in ovaries by dissecting female wasps

This experiment was conducted to determine the number of eggs produced in the ovarioles of each female wasp. Comparison of the results of this test with the results from the previous experiment indicated how many eggs were not laid by wasps.

4.3.1 Materials and methods

To examine ovariole development, 22 females of *A. subandinus* and 48 females of *O. lepidus* were held at 24°C without hosts but with honey and water, for predetermined periods ranging from 0 to 18 days and then dissected. The wasps had been reared on well fed hosts during their larval development.

In addition, females which were provided with hosts over their whole lifespan (Section 1.a), were collected immediately from the insect culture upon death. These females were dissected to count the remaining number of eggs in their ovaries. The total number of eggs produced by each female was assumed to be the sum of the number of mature eggs present in the ovaries and any eggs deposited during host encounters. It is likely that females superparasitised some of the hosts. Mature eggs were oocytes inside the oviducts positioned at the base of the ovaries, not associated with nurse cells and of full size (Flanders, 1942; Price, 1975). Mature eggs were readily distinguishable from immature ones because of their well-formed chorion and smooth surface.

The females were dissected in Phosphate Buffer Saline (PBS, 138 mM NaCl, 2.7 mM KCl, 1.47 mM KH_2PO_4, 7.3 mM Na_2HPO_4, pH 7.6), using fine forceps (INOX No. 4), by gently pulling out the tip of the abdomen including the base of the ovipositor. The ovaries were

teased out of the abdomen, placed in a drop of PBS solution on a glass slide and ovarioles separated with two minute pins. The number of mature eggs present in the oviducts and ovaries was counted using a stereomicroscope. Since the number of eggs in the two ovaries differed within each female, the eggs in each ovary were counted separately.

4.3.2 Results

The number of ovarioles observed in the reproductive system of *O. lepidus* varied from 6 to a maximum of 12 ovarioles in each ovary (Fig. 4.7.B). From 48 dissected females, 71.5% had 8 ovarioles, 12% had 6 ovarioles, 11.5% had 12 ovarioles and 5% had 9 ovarioles in each ovary (Appendix 4.1). The number of mature eggs in the ovaries increased with increasing number of ovarioles. The number of mature eggs gradually increased in nulliparous females until 10 days post emergence and gradually declined there after (Fig. 4.8).

Females of both species had a large number of eggs in their ovaries when they emerged. The oocytes that were positioned at the base of the ovaries were full size mature eggs, the other oocytes gradually became smaller towards the distal end of the ovarioles. The oocytes could be clearly seen through the ovariole tubes. *A. subandinus* females had many oocytes in various stages of maturity. Females of this species stored the mature eggs in the reservoirs of ovaries. The eggs of *O. lepidus* were aligned in a row like a chain in the ovarioles but at the base of the ovarioles 3-8 developed eggs were in 3 rows. Females of *O. lepidus* stored their eggs in the ovarioles.

Egg counts in dissected females together with number eggs laid during the females' lifetime indicated that total egg numbers can reach a maximum of 275 per female for *O. lepidus* and 215 for *A. subandinus*. In *O. lepidus* and *A. subandinus* females that had access to hosts throughout their lifetime, means of 64 and 35 eggs respectively, were counted after death (Tables 4.3 and 4.4).

Figure 4.7: Ovaries of the two parasitoids, the reproductive organs of female *A. subandinus* with 2 ovarioles in each ovary (**A**), ovaries of *O. lepidus* with 6-12 ovarioles per ovary (**B**).

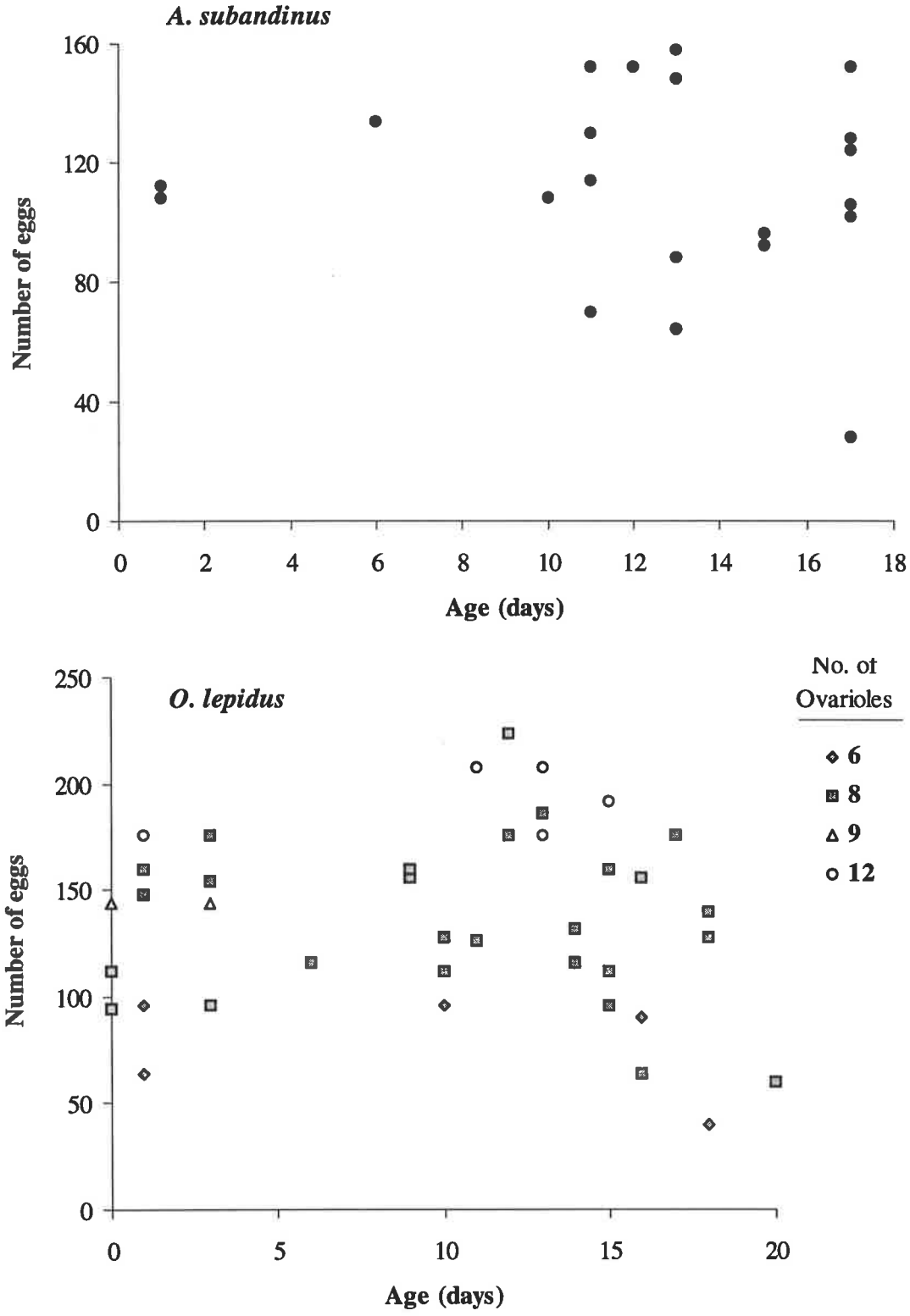


Figure 4.8: The number of mature eggs in *A. subandinus* females (n= 22) and *O. lepidus* females (n= 48) at various ages. These females had not previously laid egg (i.e. were nulliparous).

Table 4.3: Estimate of lifetime egg production: the number of eggs in *O. lepidus* ovaries after egg-laying at the end of life-time, and the number of eggs laid during the lifetime of a female.

No. of dissected female	No. of ovariole per ovary	Host density	Eggs laid	Age of death	Remaining eggs	Total
1	12	20	159	18	56	215
2	8	20	71	8	153	224
3	8	40	143	9	64	207
4	8	80	191	14	48	239
5	8	160	235	10	28	263
6	12	160	243	12	32	275
Mean			*		64	237

* Mean eggs laid varied in different density

Table 4.4: Estimate of lifetime egg production: the number of eggs in *A. subandinus* ovaries after egg-laying at the end of life-time, and the number of eggs laid during the lifetime of a female.

No. of dissected female	No. of ovariole per ovary	Host density	Eggs laid	Age of death	Remaining eggs	Total
1	2	20	81	15	62	143
2	2	20	117	12	28	145
3	2	40	111	16	24	135
4	2	80	167	13	43	210
5	2	160	199	12	16	215
6	2	160	182	10	20	202
Mean			*		35	170

* Mean eggs laid varied in different density

4.3.3 Discussion

The number of ovarioles per ovary varies in the Hymenoptera and in some species of *Apanteles*, the ovary and its oviduct are modified for egg storage (Flanders, 1950; Price, 1975; Dowell, 1978 and Jervis and Kidd, 1996). The reproductive system of female *A. subandinus* is similar to that of other members of the genus *Apanteles* (Flanders, 1942), and consists of

two ovaries each with two ovarioles that open into an enlarged reservoir and common oviduct (Fig. 4.7.A). The common oviduct opens into the base of the ovipositor.

The number of mature eggs available for oviposition increased from 3-5 per ovary in newly emerged *A. subandinus* females to 54 per ovary after one day. *O. lepidus* females had 3-7 mature eggs per ovariole on the first day after emergence which increased to an average of 9 per ovariole after one day. Oviposition in *O. lepidus* and *A. subandinus* occurs shortly after emergence. Differences in numbers of eggs recorded at different ages showed that egg production was continuous throughout the female's lifetime (Appendix 4.1).

In many species of Hymenoptera, there is a positive correlation between the number of eggs produced by parasitoid females and the size and number of ovarioles in their ovaries (Nealis *et al.*, 1984; Le Masurier, 1987 and Jervis and Kidd, 1996). Results from this trial agree with previous investigations on other parasitoids, and confirmed that the number of eggs produced by *O. lepidus* was higher in females with more ovarioles (12 ovarioles: mean 192 eggs) than those with fewer ovarioles (6 ovarioles with mean 77 eggs).

4.4 Temperature and fecundity

Ambient temperatures are known to affect the rate of increase of parasitoid populations (Powell and Bellows, 1992; Van Steenis, 1993). This study aimed to measure the lifetime fecundity of the two parasitoids at 5 constant temperatures.

4.4.1 Materials and methods

Both hosts and parasitoids were taken from insects cultured in the insectary (Section 2.2). Potted potato plants approximately 20cm high, were infested with 50 newly emerged 1st instar PTM larvae and left for 24hr in the culture room. After this period, the infested plants were placed in cages (33 × 26 × 26cm) provided with a perspex top and sides, and gauze door.

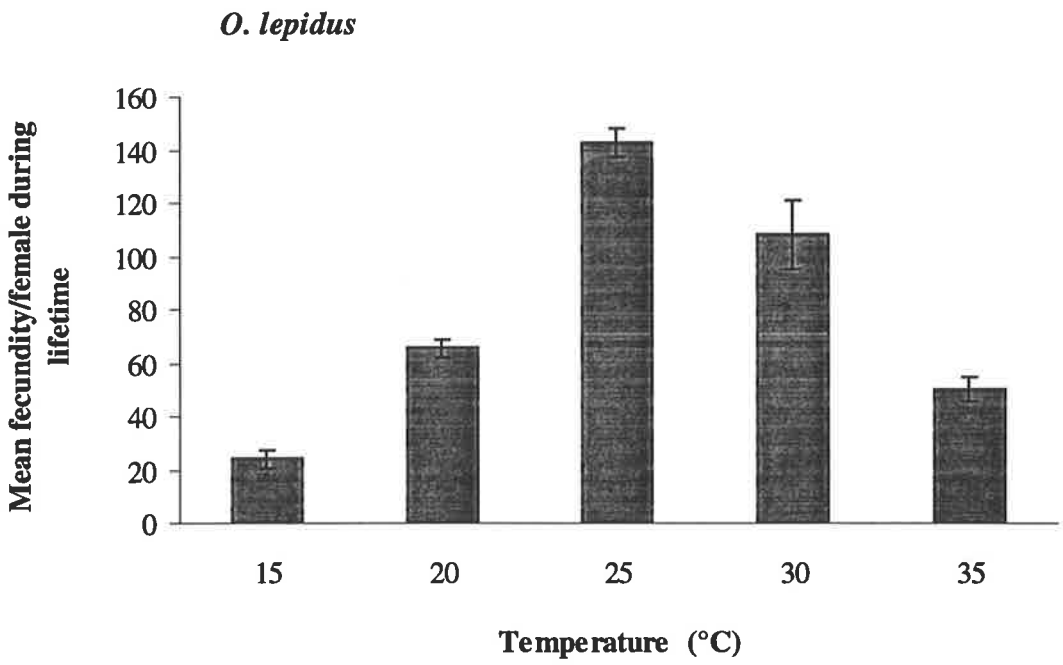
Honey solution (20%) was provided as the food source. The cages were transferred into incubators with constant temperatures of 15°C, 20°C, 25°C, 30°C and 35°C under continuous light. A newly emerged gravid female parasitoid of either species was released into each cage individually and allowed to parasitise the PTM larvae for 24hr. Infested plants were replaced every day for the lifetime of the wasp.

This experiment was replicated three times for each parasitoid species individually. The data from this experiment were analysed by regression. The number of hosts parasitised by each wasp was recorded and a histogram fitted individually for each species to show the total number of progeny produced per female at each temperature. The net reproductive rate (R_0) was estimated for the two parasitoids at different temperatures using the equation described in Section 4.2.1a.

4.4.2 Results

The mean fecundity was highest at 25°C for *O. lepidus* and at 30°C for *A. subandinus* (Fig. 4.9). The mean of total hosts parasitised by *O. lepidus* females at 15°C, 22°C and 35°C were significantly lower than that at 25°C. Fecundity of *A. subandinus* females was much less at 15°C than the higher temperatures from 25°C to 35°C. Females of *O. lepidus* laid more eggs than females of *A. subandinus* except at 35°C. Longevity of female *O. lepidus* was less at high temperature, but the mean of daily fecundity was greater at high temperature than low temperature where wasps were alive longer (Fig. 4.10).

The average number of eggs deposited by each female per day formed a skewed-right pattern and peak of oviposition appeared at 2-4 days after emergence at most temperatures. Adults continued laying eggs until death. The maximum net reproductive rate occurred at 25°C for



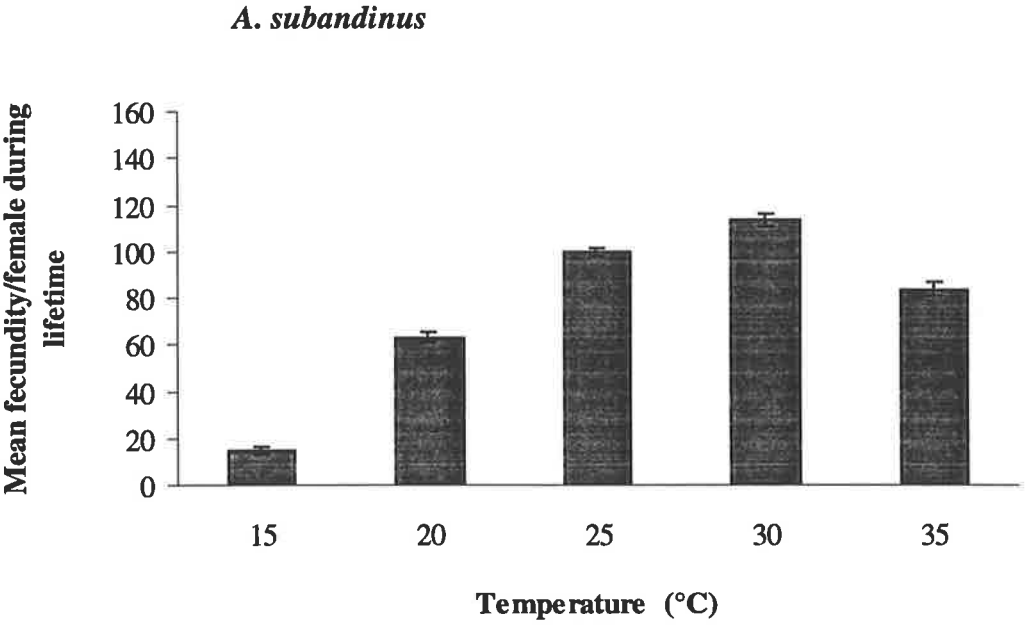
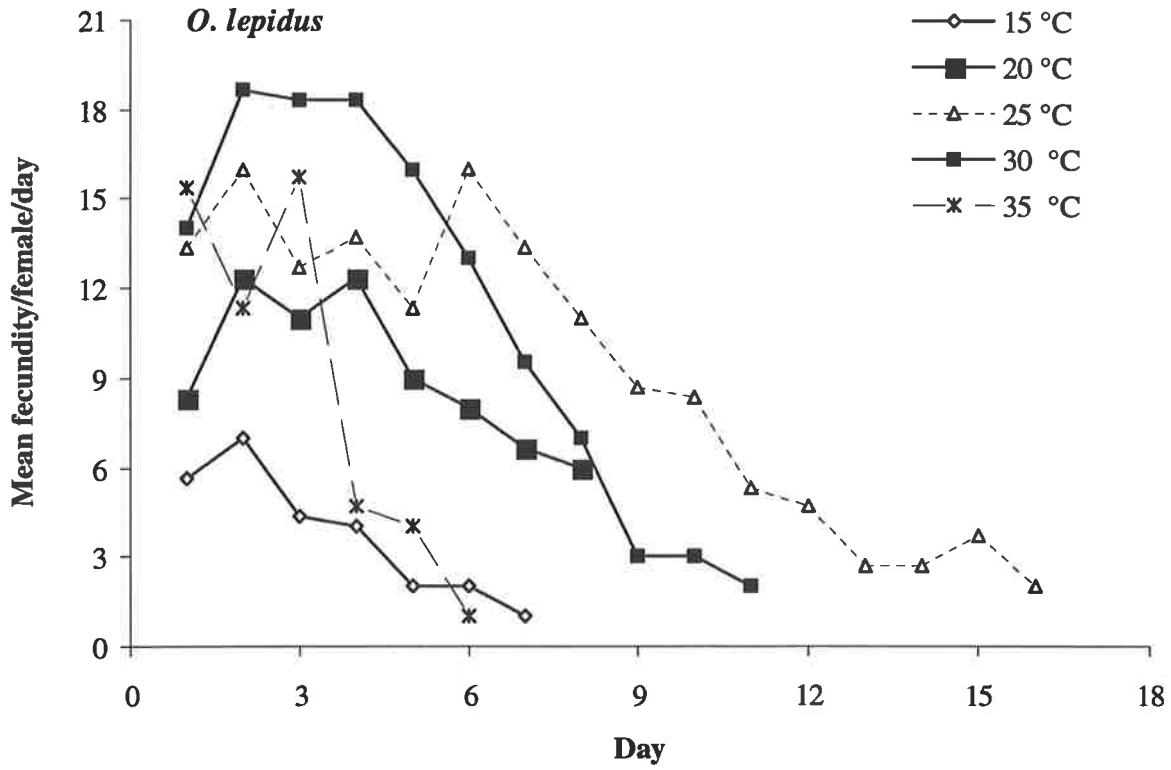


Figure 4.9: Comparison of the mean fecundity of *A. subandinus* and *O. lepidus* at 5 constant temperatures. Error bars indicate ± standard error of means, n= 3 females per species per temperature.



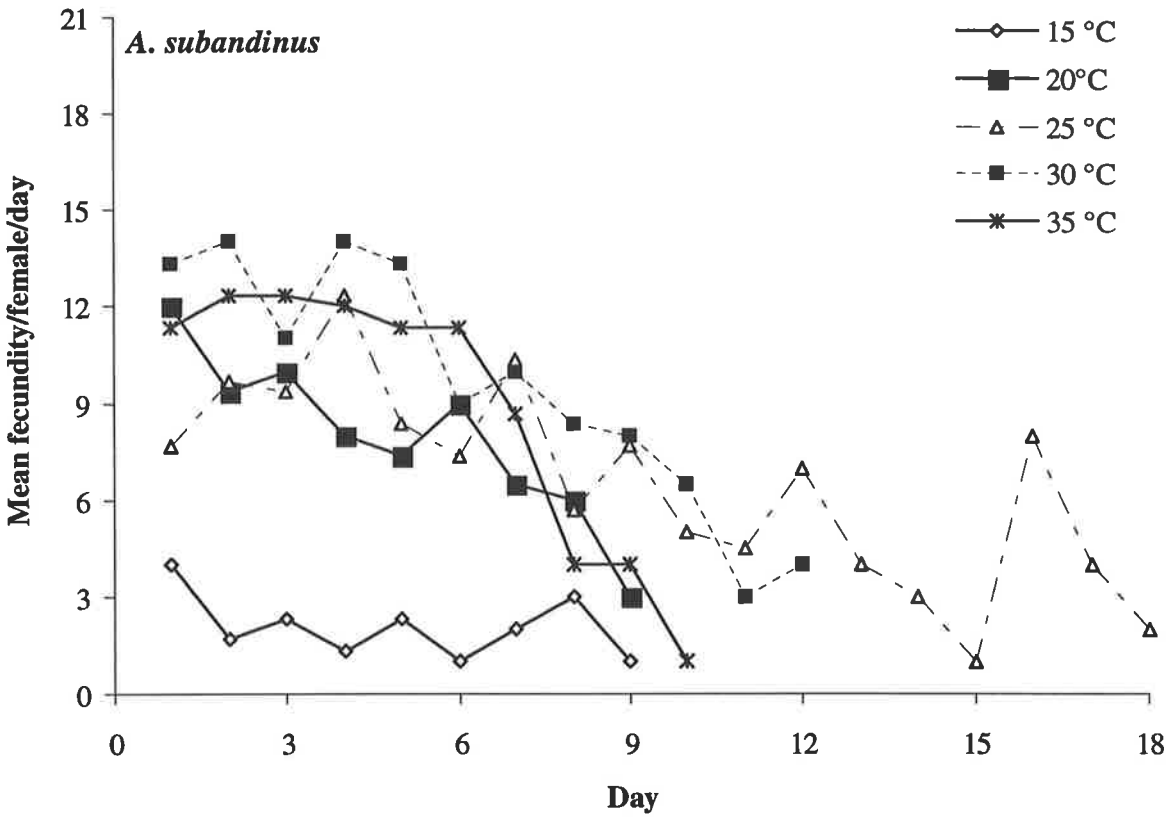


Figure 4.10: Mean daily reproduction (egg/female/day) by two parasitoids at 5 constant temperatures in the incubators. n= 3 for each species at each temperature.

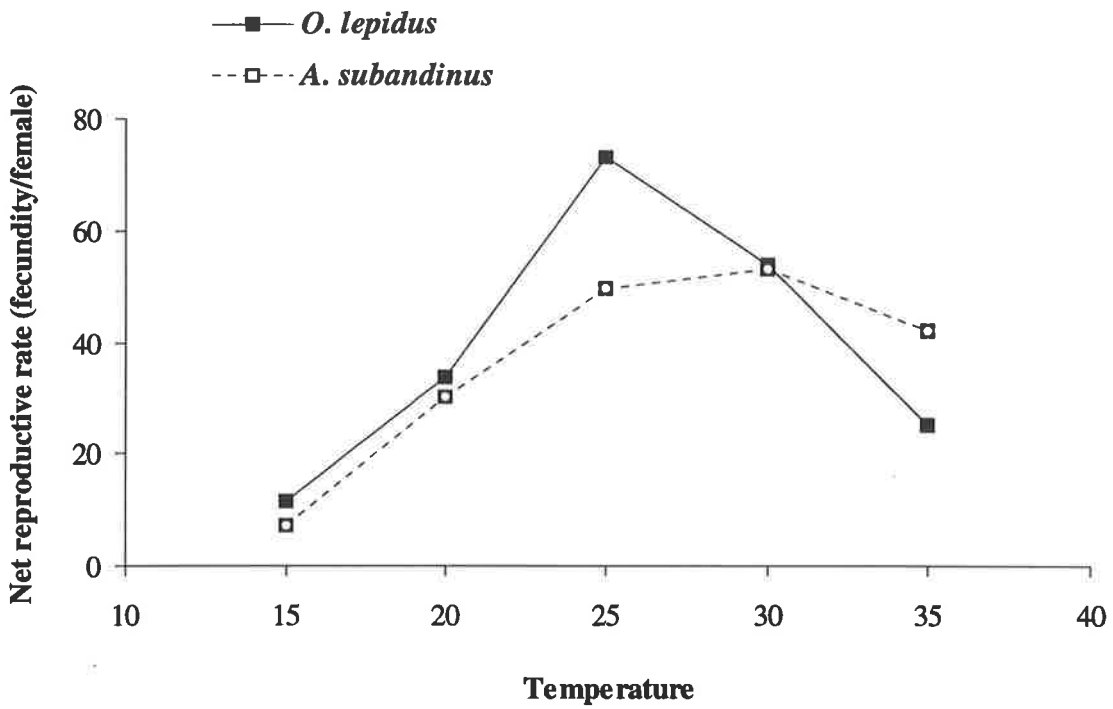


Figure 4.11: The net reproductive ability (R_0) of *A. subandinus* and *O. lepidus* at five constant temperatures. n= 3 females/species/temperature.

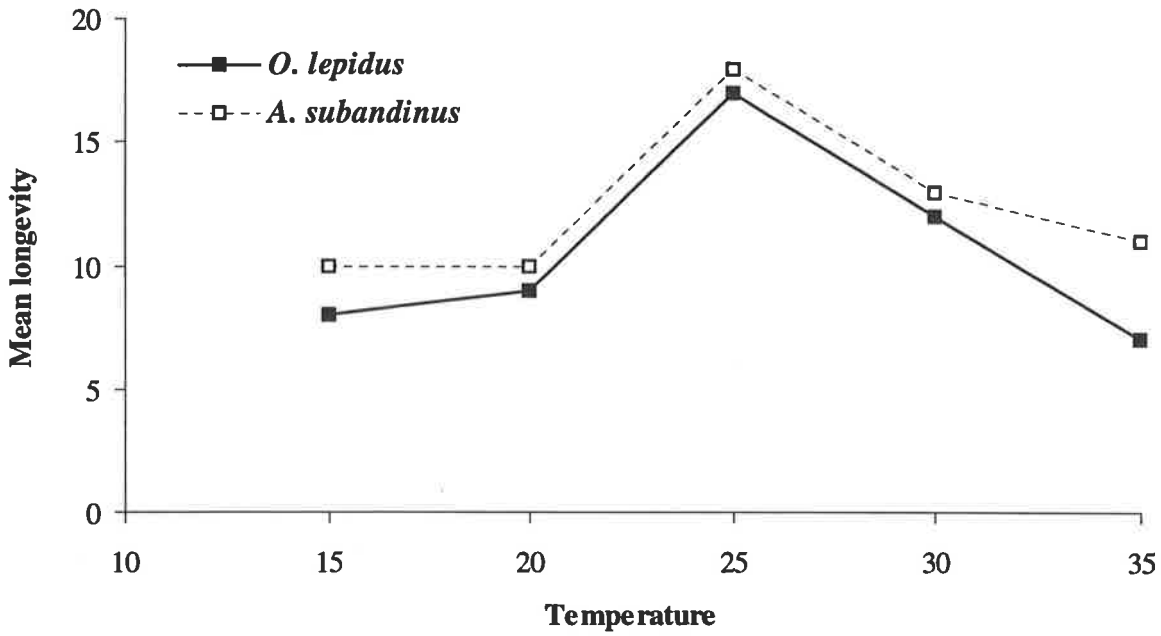


Figure 4.12: Mean longevity of *A. subandinus* and *O. lepidus* females that laid eggs throughout their lifetime. n=3 females/species/temperature.

O. lepidus and at 30°C for *A. subandinus* (Fig. 4.11) while longevity was greater at 25°C (Fig. 4.12).

4.4.3 Discussion

Oviposition by the two parasitoids occurred at all temperatures in the range 15-35°C from the first day, peaked after 1-2 days and then decreased gradually with age. The optimal temperature for egg laying was 30°C for *A. subandinus* and 25°C for *O. lepidus*. Low and high temperatures reduced the egg laying activity of both species.

Females of both species lived for a shorter time and laid fewer eggs at 15°C than at other temperatures. This confirmed Messenger's findings (1968), who found that low temperatures greatly decreased the rate of behavioural activities. While the egg load of female *O. lepidus* is higher than *A. subandinus*, there was no significant difference between the rate of oviposition by the two species. A temperature of 35°C reduced egg-laying by *O. lepidus* more than by females of *A. subandinus*. The results confirmed previous results on the development of the two species (Section 3.4) that *A. subandinus* is stronger than *O. lepidus* at 15°C and 35°C.

4.5 Field sampling

Field sampling, as part of the investigation of reproduction of the two parasitoids, had the following aims:

1) quantifying populations of the two parasitoids to show their relative importance in two major potato growing areas in South Australia;

2) comparing field populations of the two parasitoids to the laboratory population with respect to sex ratio and adult size (Sections 4.2.5 and 4.2.6).

4.5.1 Materials and methods

Sampling was carried out in two potato growing areas of South Australia, namely, the Adelaide Hills (Woodside, Charleston, Balhannah, Hahndorf, Lobethal, Birdwood and Lenswood) and Virginia (Angle Vale). Infested foliage was collected from pesticide-free crops in each field between early January and May. Field sampling was organised once every two weeks. A sweep-net was used to collect foliage of potato plants and infested leaves were separated from those collected with the net. Numbers of samples were 40 sweeps on each date and a total of 400 during one growing season in each area. The collected foliage was kept in the insectary and larvae were reared in rearing boxes provided with potato tubers for larva feeding and sand for their pupation (Section 2.2). The percentage of each parasitoid in the PTM population was estimated from the total number of recorded adults.

4.5.2 Results

The observation from irregular sampling indicated that potato crops are infested with PTM at Virginia and the Adelaide Hills and the two parasitoids are established in both areas. The populations of the two parasitoids and PTM were higher at Virginia than in the Adelaide Hills (Table 4.5). This may have been due to a more favourable environment. However, the number of insecticide applications in the Virginia area is more than in the Adelaide Hills (Personal communications with farmers). Certainly, this relative abundance of overall parasitism may change during the year, so these results are indicative only of the period sampled.

Table 4.5: The results of field sampling parasitoids of PTM during four months in the Adelaide Hills and Virginia the two potato growing areas of South Australia.

Growing regions	First sample	Last sample	Total insects	% Parasitised PTM			% Unparasitised PTM
				A. subandinus	*O. lepidus*	*Copidosoma sp.*	
The Adelaide Hills	10.2.96	27.05.96	695	15.3	5.5	11.8	67.5
Virginia	05.01.97	05.03.97	1211	10.2	8.3	11.1	70.6

4.5.3 Discussion

The limited results suggested that conditions in potato growing regions in South Australia are more favourable for *A. subandinus* than for *O. lepidus*. The same results were obtained by Briese (1981) in Virginia, South Australia and by Whiteside (1981) in Transval Highveld, South Africa, when these researchers estimated parasitism of PTM. Pam Strange (1993, unpublished data), reported inverse results for *A. subandinus* and *O. lepidus* from sampling in some potato growing sites of Virginia at July 1994. Higher percent parasitism by *A. subandinus* than *O. lepidus* in potato growing regions in South Australia especially in the Adelaide Hills showed that the first species is better adapted to this area. Although, *O. lepidus* can oviposit in more hosts and develops more quickly than PTM, it seems this parasitoid is not present in large numbers in the early season temperature and is thus poorly synchronised with PTM at the beginning of season.

4.6 Sex ratio

It is a great advantage to a species to have an adjustable sex ratio (Jackson, 1966). Several mechanisms for the adjustment of sex ratio by parasitoids have been identified (Flanders, 1965; Wylie, 1973). King (1987) reviewed the factors determining the final sex ratio of

emerging wasps for 100 species distributed among 16 families. These factors are classified into four groups: paternal characteristics, host characteristics, environmental characteristics and factors influencing local mate competition. Waage *et al.* (1985) discussed several factors that might influence the sex ratio of insects in culture. When a parasitoid was reared for several generations in the laboratory, it lost sex alleles during inbreeding (Green *et al.*, 1982; Southamer *et al.*, 1992). In addition, sex ratios can be adjusted by scramble competition, in which one sex has a higher incidence of mortality under conditions of superparasitism (Flanders, 1965; Waage and Ng, 1984).

The aim of this trial was to compare sex ratio between laboratory and field populations within the two parasitoid species.

4.6.1 Materials and methods

To estimate sex ratio of *A. subandinus* and *O. lepidus*, the adult males and females were counted in each parasitoid reproductive test, described in section 4.2.1; the PTM larvae were reared after being exposed to parasitoids and the sex was determined after adults emerged.

To measure sex ratios from field populations, the two species of parasitoids were collected from two potato growing regions in South Australia, Virginia and the Adelaide Hills. The numbers of males and females were recorded using two methods of collecting in the field. Firstly, a sweep-net was used to collect adults from the upper parts of the plants. Secondly, foliage mined by PTM larvae was collected and the larvae reared in the insectary to obtain the adult parasitoids.

The Chi-square test (Zar, 1984) was performed to analyse difference between the number of males and females recorded for both insectary and in the field and also their sex ratio for the two parasitoids.

4.6.2 Results

Data obtained from both the insectary and field indicated that the percentage of males of both species was higher than females (Table 4.6). The ratio of males to females increased slightly for both species when wasps were reared in the insectary. Sex ratio was significantly male biased for the two parasitoids only in the insectary, but there was no significant differences between sex ratio of them in both insectary and in the field (Chi-square test).

Table 4.6: Comparison between sex ratio of *A. subandinus* and *O. lepidus* in the insectary and in the field.

Parasitoid	**Insectary**				**Field**			
	*n	Male	Female	Sex-ratio	*n	Male	Female	Sex-ratio
A. subandinus	635 (21)	415	220	0.65	123 (18)	72	51	0.59
O. lepidus	1514 (27)	980	593	0.62	101 (13)	55	46	0.54

* Total number of insects and (number of samples)

4.6.3 Discussion

A. subandinus and *O. lepidus* produce more males than females in both the insectary and in the field. The number of males produced by *A. subandinus* females increased with increasing number of generations in the insectary, and this change was much slower for *O. lepidus*. It should be noted here that laboratory rearing affects the behaviour and reproductive fitness of these species, particularly in *A. subandinus* after 4-5 generations.

No cytological investigation has been made of this insect, and it is presumed that males *A. subandinus* are haploid and arise from unfertilised eggs. Southamer *et al.*, (1992) found that diploid males occur in several Braconid and Ichneumonid species and they have a low fertility and are more difficult to establish than taxa with other modes of sex determination. They believed that laboratory rearing influenced the number of sex alleles and consequently

production of diploid males could obstruct biological control severely if appropriate precautions are not taken. It is possible that inbreeding caused changes in the sex ratio of *A. subandinus* and increased the frequency of males in the insectary culture.

4.7 Parasitoid size

When population density becomes high, reduced body size and low fecundity can cause a decrease in population density, and this is one of the most important mechanisms of population regulation in some insect species (Dempster, 1975).

It was casually observed that the size of males and females of *A. subandinus* reduced with number of generations reared in the insectary. However, this size change was not apparent in *O. lepidus*. Thus, only *A. subandinus* was measured in a comparison to insectary reared versus field captured wasps.

4.7.1 Materials and methods

The antennae and body length of 20 pairs of *A. subandinus* adults reared in the insectary after more than 6 generations were compared with pairs emerged from larvae collected in the field. Antenna length was measured from the tip of the antenna to the base of the pediculus and body length from the base of antenna to the tip of the abdomen. Measurements were made using a stereomicroscope with a ruler in millimetres. Adult wasps were placed in a vial and put in an ice box for 10 minutes to immobilise them before measuring one at a time under the stereomicroscope. All data were analysed by analysis of variance (ANOVA) using Genstat 5 (Lane and Payne, 1996).

4.7.2 Results

The results indicate differences in adult sizes between the wasps emerged from the insect culture and those collected from the field (Table 4.7). The size of the body and antenna

decreased when insects were reared for 4-5 generations in the insectary. As can be seen from the data, the ranges were greater in reared insects than in those collected from the field.

Table 4.7: Comparison of antenna and body lengths of male and female *A. subandinus* reared in the insectary with wasps collected from the field.

Location	Male				Female				n
	Antenna*		Body*		Antenna*		Body*		
	Range	Mean*	Range	Mean*	Range	Mean*	Range	Mean*	
Insectary	3.2-3.8	3.5± 0.3	2.1-3.2	2.7± 0.3	2.1-2.66	2.4± 0.2	2.4-3.5	2.9± 0.4	20
Field	3.5-4.1	3.7± 0.2	2.8-3.2	2.9± 0.1	2.3-2.8	2.5± 0.2	2.9-3.3	3.1± 0.2	20

*Length is in mm **Mean ±sd.

4.7.3 Discussion

The adult body sizes of *A. subandinus* collected from the field were larger than adults reared in the insectary. Researchers described the relationship between adult parasitoid size and the quality of their host, superparasitism and their sex ratio (see Godfray, 1994 and Jervis and Kidd, 1996 for more information). During this study many observations on *A. subandinus* rearing indicated that the small size of adults occurred as the sex ratio gradually changed after 3-4 generations.

4.8 General Discussion

The results of different egg-laying tests of *A. subandinus* and *O. lepidus* showed high variability of egg-laying under different conditions. In this study, the number of eggs laid per day by both parasitoids was controlled by at least two variables; host density and temperature. Although, egg production of the two species is variable and depends to their size and number of ovarioles in their ovaries.

Price (1975) stated that the major limitation of fecundity of parasitoids is the rarity of hosts. The results of oviposition by the two species indicated that any increase in the density of the host caused a proportional increase in the parasitism of the host. The results are in agreement with DeBach and Smith (1941) who reported that the number of progeny of a parasitoid will depend upon how many hosts the parasitoid can find, and this depends upon the density of its host population.

Temperature has a significant influence on the egg-laying of females of the two parasitoids. In the laboratory, oviposition by both parasitoids varied with temperature (15°C to 35°C). Changes in temperature affect the reproduction of an insect parasite and the resulting mortality of its host by affecting the rate at which insect parasites develop and reproduce. It might be concluded that the optimal temperature for fecundity of *O. lepidus* was 25°C and for *A. subandinus* was 30°C.

Egg production was not a constraint on the maximum number of parasitoid progeny produced per day. Despite the availability of abundant and suitable hosts for oviposition, females of both species did not deposit many of the available eggs. Average egg deposition by a female of *A. subandinus* and *O. lepidus* per day was 13.5% and 15% respectively of the available eggs in their ovaries. Dissections showed that females had mature eggs from the first day but, they could release these eggs if suitable conditions was provided.

The average female's life span in both species was shorter when they were exposed to a high number of hosts than when exposed to a low number of hosts. Females laid more eggs at the beginning of their life (first 3-4 days) than the end. Thus, maximum egg-laying was not related only to long longevity of females.

[IC2]Developmental times of *O. lepidus* and *A. subandinus* estimated from chapter 3, showed that the potential number of generations per year could be 15-18 in *A. subandinus* and 13-15

in *O. lepidus* at temperatures between 25 and 30°C in laboratory conditions. As the number of annual generations of the PTM is 10-20 in the same conditions, *A. subandinus* and *O. lepidus* can reproduce 1.4 and 1.2 times during one host generation, respectively. This is an advantages of the two species to regulate host populations.

The proportion of males produced by *A. subandinus* and *O. lepidus* under experimental conditions was higher than collected from the field. Since in all experiments only newly emerged PTM larvae were exposed to parasitoid females, there was no correlation between the age and size of the host with parasitoid sex ratio. In fact, under constant experimental conditions, progeny sex ratio variations in both *A. subandinus* and *O. lepidus* occurred due to factors such as number of generations in culture and where presumably diploid males occurred.

The results of different experiments in this study clearly indicated *A. subandinus* and *O. lepidus* produce fewer progeny than the results reported by Cardona and Oatman (1975) and Oatman *et al.* (1969). This disagreement might be due to different factors such as environmental conditions, nutrition, genetic events or genetic drift which altered the behaviour and fitness of the two parasitoids and their reproductive adaptations (e.g. Geden, *et al.*, 1992). The maximum number of egg laid (i.e. 243 eggs) by a female *O. lepidus* in optimum conditions for its lifetime provided with a high density of hosts, including the remaining eggs in its ovaries after death (i.e. 32 eggs) was 275 eggs, whereas Oatman *et al.* (1969) reported a maximum of 791 eggs for *O. lepidus* during the lifetime of a female.

In conclusion, the results of the different experiments showed following advantages of the two parasitoids when their reproductive capacity is compared:

1) fecundity of female *O. lepidus* is higher than *A. subandinus* in different densities of PTM at 24°C,

2) female *O. lepidus* has bigger body size and ovaries and a greater number of ovarioles that allow it to produce more eggs than *A. subandinus*,
3) female *O. lepidus* does not decrease in size in laboratory, but *A. subandinus* becomes smaller when kept in continuously laboratory culture,
4) *A. subandinus* increases its male sex ratio after generation in the laboratory, but this is less apparent with *O. lepidus*,
5) fecundity of female *A. subandinus* is higher than *O. lepidus* at high temperature,
6) *A. subandinus* has the advantages of shorter developmental time and more generations per annum than *O. lepidus*,
7) in both species, there is essentially no preoviposition period.
8) the male ratio of both species under natural conditions (field), was less than insects reared in the insectary,
9) both species have greater fecundity than their host.

In general, the high fecundity and highly efficient egg laying of *O. lepidus* are more adapted to a high density of PTM than *A. subandinus*. As previous results (Chapter 3) indicated, *A. subandinus* is better adapted to very low temperature than *O. lepidus*, therefor, it could be suggested again that releasing of *A. subandinus* at early of the growing season with low host populations would increase the ability of biological control of PTM in the field.

Chapter Five

Host finding and responses of the two parasitoids to plant/host complex in the wind tunnel

Wind tunnel with experimental apparatus

"Parasitoids have evolved and function within a multitrophic context and their physiology and behaviour are influenced by elements from other trophic levels such as host and its plant food"

P. W. Price, 1980

5.1 Introduction

Host finding by a parasitoid depends on responses to cues from the host habitat and the host itself (Doutt, 1964; Vinson, 1975). Salt (1935), Doutt (1959) and Vinson (1975) state that host-finding by a parasitoid consists of five steps: 1) host-habitat-location, 2) host-location, 3) host acceptance, 4) host suitability and 5) host regulation.

Understanding of factors influencing the searching behaviour of parasitoids has advanced substantially in recent years (see Vet and Dicke, 1992). There is considerable evidence that some parasitoids use chemical stimuli associated with interactions between the host and its food plant and respond to host herbivore-induced plant volatiles (Turlings *et al.*, 1990, 1991; Vet and Dicke, 1992; Tumlinson *et al.*, 1993; Keller and Horne, 1993; Steinberg *et al.*, 1993; Takabayashi *et al.*, 1994). Odours from hosts, host products or host plants can be important cues used by parasitoids to locate the habitats of their hosts and host themselves (Keller and Lewis, 1989; Vinson, 1991; Vet and Dicke, 1992; Agelopoulos and Keller, 1994a&b; Kitt and Keller, 1998).

Little is known about the role of chemical cues in the foraging behaviour for PTM parasitoids. Hendry *et al.* (1973) reported that host-finding by female *O. lepidus* was mediated by two kairomones present in the frass of the host. They found that the active volatile component in the frass, heptanoic acid, normally functions to elicit only intense tapping by antennae and directed movements by the parasitoid females and that the probing response is mediated by the non-volatile component upon direct contact with the faeces. Keller and Horne (1993) studied searching and host finding behaviours by *O. lepidus* in a wind tunnel and they found that although female *O. lepidus* flew to both undamaged and mechanically damaged potatoes, more females flew to damaged plants when given a choice. Their research indicated that *O.*

lepidus can discriminate between the volatiles of a mechanically damaged potato plant and those of a potato plant damaged by PTM larvae.

There are no reports on the foraging and host-finding behaviour of *A. subandinus*, nor is there any data available on the efficiency of *A. subandinus* and *O. lepidus* if reared on PTM fed on potato tuber, then exposed to hosts on other Solanaceous plants. Sheehan and Shelton (1989) tested *Diaeretiella rapae* (Hymenoptera: Aphidiidae) a parasitoid of aphids on cruciferous plants. They reared and experienced wasps on one host plant (collard) and released to other host plant (potato) infested with host. These researchers found that postemergence experience with potato did not increase orientation to potato, but postemergence experience with collard resulted in a trend of increased likelihood of flying to collard odour.

A better understanding of the host-finding behaviour of *O. lepidus* and *A. subandinus* may play an important part in developing a more effective biological control program for PTM. Chemical cues could be used to stimulate parasitoids' host-finding, e.g. in rearing or prior to release of wasps. Therefore, this chapter examines the cues that lead *A. subandinus* to its host, and the responses of the two parasitoids to different host plants of PTM. Two series experiments were conducted for the two parasitoids in the wind tunnel. Both were mainly concerned with step two of the host finding process, host location.

5.1.1 Aims

The aims of this study were:

1) to determine which factor (s) female *A. subandinus* utilise when foraging for hosts,

2) to determine the role of host plants in affecting the behaviour of *O. lepidus* and *A. subandinus* in the process of host finding.

5.2 General materials and methods

Parasitoids and their release

Parasitoids of both species were 2-3-day-old females and reared on PTM larvae feeding on potato tubers in the insectary (Section 2.2). Prior to their use in experiments, females were kept in cages with males where they had access to honey and water at all times.

Inexperienced wasps vary strongly with regard to the start of their host-searching behaviour and rarely fly to plants when released in the wind tunnel. In contrast experienced wasps start searching almost immediately. One of the most important factors in research on host-parasitoid systems involves prior experience of the parasitoid which modifies their foraging behaviour (Vet and Dicke, 1992). A screened cage (15 × 15 × 20cm) containing infested potato leaves with 10 PTM larvae was placed in the wind tunnel and five wasps (in each trial) were released in the cage for 30-60min prior to release in the wind tunnel to gain experience with hosts plants and host insects. Thus, all parasitoids used in tests were 2-3-day-old and experienced before flying in the wind tunnel. All tests were carried out between 9am to 6pm.

The latency of flight (the time from wasp placement in the release vial to take off) and flying time were recorded during tests, as was the site of landing. The behaviours were recorded using an Observer Version 2 (Noldus, 1991) and recording started when each wasp oriented towards odours the plants and finished when each wasp landed on one of the two plants.

Assays in a wind tunnel

The wind tunnel used in these experiments was a rectangular plexiglass chamber (65 x 65 x 160cm) described by Keller (1990). The environmental conditions in the wind tunnel were the same for experiments in both sections: wind speed 32.4cm/s, temperature 24°C, light

intensity 4800 lux at the insect release site.

Individual wasps were released 25cm above the floor of the wind tunnel at the same height as the target plants. They were released from a glass tube in size 2.5cm in diameter and 13.5cm in length with two open ends. The vial was placed horizontally in the air-stream on a wooden stand and positioned so that the odour plume could pass through. The distance from the releasing vial to the plants was 30cm.

Each female had a time limit of five minutes to respond. Wasps that landed on the walls or ceiling were omitted from the analysis. The number of females that responded in each cohort and choice of landing site was recorded.

The plants (damaged/undamaged, potato/tomato or potato/eggplant) were placed 10cm apart (2cm between their lateral leaves). A smoke test using a mixture of acetic acid and diaminoethane showed that the two plants produced separate plumes.

Data analysis

The numbers of females attracted to each of the paired plants were compared by a Binomial test (Zar, 1984), using a computer program written by M. Keller in 1997. In all tests $P< 0.05$ was used to determine significance.

5.3 Responses of *A. subandinus* to its host (PTM) and host plant (Potato)

The ability of *A. subandinus* females to discriminate between two types of damaged potato leaves was studied to determine if *A. subandinus* could distinguish between the plant-host complex and a mechanically damaged plant.

5.3.1 Materials and methods

Potato shoots with at least three matured leaves of the same size and as similar as possible in shape were cut with a surgical knife and fixed in containers (Section 2.3). In each test, two container plants were used, a potato infested with 10 48hr-old larval PTM and a mechanically damaged potato. The second plant was damaged just 1hr prior to test using a pin to pierce the leaves similar to the shape of damage caused by PTM.

Two experiments were conducted. In the first experiment, 51 female *A. subandinus* were released individually, each only once, in the wind tunnel. In the second, 65 female wasps were released individually twice. At the 2nd release, the positions of the two plants were switched. The aim of the second experiment was to be confident that wasps responded to separate plumes not to one direction.

5.3.2 Results

Female *A. subandinus* were strongly attracted to infested plants when given a choice between potato infested by PTM and potato mechanically damaged in both series of tests (Fig. 5.1.A&B). There were highly significant differences between the two groups of females ($P \leq$ 0.001, binomial test) which had chosen infested plants with PTM and those that had chosen mechanically damaged plants. A small proportion (23% in the first series and 17% and 8% respectively in the 1st and 2nd release of the second series of tests) flew to the sides of the wind tunnel.

The wasps walked around the inside and outside of the vial after have being placed into the releasing vial, then extended their antennae away from the face and raised them. After a period of standing and moving, they finally flew to the plants. On a few occasions some flew

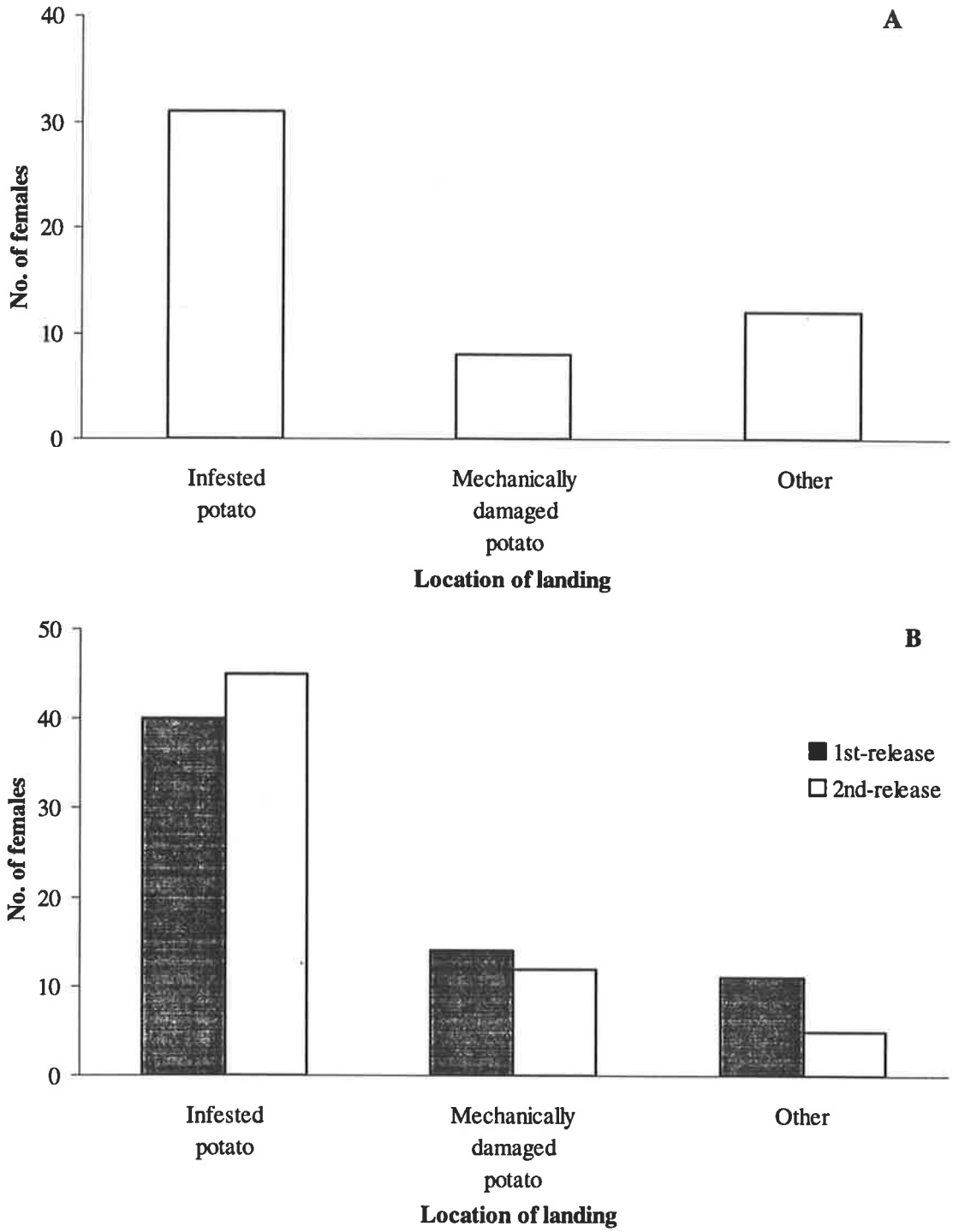


Figure 5.1: Landing sites of *A. subandinus* females when given a choice between potato plant infested by PTM and mechanically damaged potato in the wind tunnel. "Other" includes walls of the wind tunnel. **A)** females were released once (n = 51, $P < 0.01$), and **B)** each female was released twice (n = 65, $P < 0.01$), Binomial test.

immediately to the top of the wind tunnel. This latency of flight averaged 104.2 ± 6.0s (n= 65, range, 30-320s). The duration of latency did not differ among wasps landing in different locations The duration of flight ranged between 1-10s and the mean was 2.5 ± 0.23s. Flights were often direct and rapid toward the plants. The flight time decreased when females flew to the walls of the wind tunnel (1.7 ± 0.19s).

5.3.3 Discussion

The results show that complex cues from the larvae mining inside the potato tissues stimulate searching by *A. subandinus* females. However, this study did not separate the volatile components which attract this species. Keller and Horne (1993), similarly found that *O. lepidus* females respond to odours caused by PTM larvae by landing preferentially on infested plants.

Female *A. subandinus* discriminate between the volatiles of a mechanically damaged plant and those of a PTM larvae-damaged plant. Mattiacci *et al.* (1994) found that artificially damaged plants produce fewer compounds and in lower amounts than plants damaged by a host larva. In the present study it is possible that differences in the release rates of compounds in both potato plants damaged by host and mechanically damaged may explain the differences in response. In addition, the variability in the mix of compounds in the odour blend may result in subtle differences in the behaviour of parasitoids (Mattiacci *et al.*, 1994).

5.4 Host plant preference by the two parasitoids

Laboratory studies using olfactometers have shown that host finding by parasitoids is related to several conditions including odours from the plant upon which the host is feeding, from the host itself and products of the plant-host complex. All of these released chemicals can serve as mediators of host location (Vinson, 1976; Lewis and Martin, 1990; Prokopy and Lewis,

1993). Females of parasitoids may modify their behaviours on the basis of prior experience on a host plant (Vet and Dicke, 1992; Vet *et al.*, 1995).

The aim of this study was to determine whether responses of *A. subandinus* and *O. lepidus* females are dependent on rearing conditions (on potato tuber). The information about source of cues responsible to host finding of the two parasitoids would be useful to argue their role in the biological control of PTM.

5.4.1 Materials and methods

Potato buds and seedlings of tomato and eggplant were planted in black plastic pots size 10cm in diameter (Section 2.3). The plants for each test were approximately the same size (12-15cm) and as similar as possible in shape and surface area of their foliage. Because of differences in size of foliage of three plants with different morphological characteristics, size of plants foliage was measured using a Paton Electronic planimeter (Pearcy *et al.*, 1989). Ten cuts of foliage of each plant were measured as representative samples for choosing the same approximate size of three plants to be infested with PTM.

Tests were conducted comparing the preference for potato vs tomato and potato vs eggplant. Each plant was infested with the same number of PTM larvae (10/plant) at the same time. Each test was conducted individually for each parasitoid species. In each test, five female wasps were tested individually on the same set up plants. The number of female parasitoids released in different treatments were not similar.

5.4.2 Results

There were no significant differences in the number of *A. subandinus* females landing on the two targets in choice tests between infested potato and infested tomato leaves (P= 0.419) or

Figure 5.2: Number of *A. subandinus* and *O. lepidus* females landing on targets in two-choice experiments in the wind tunnel.

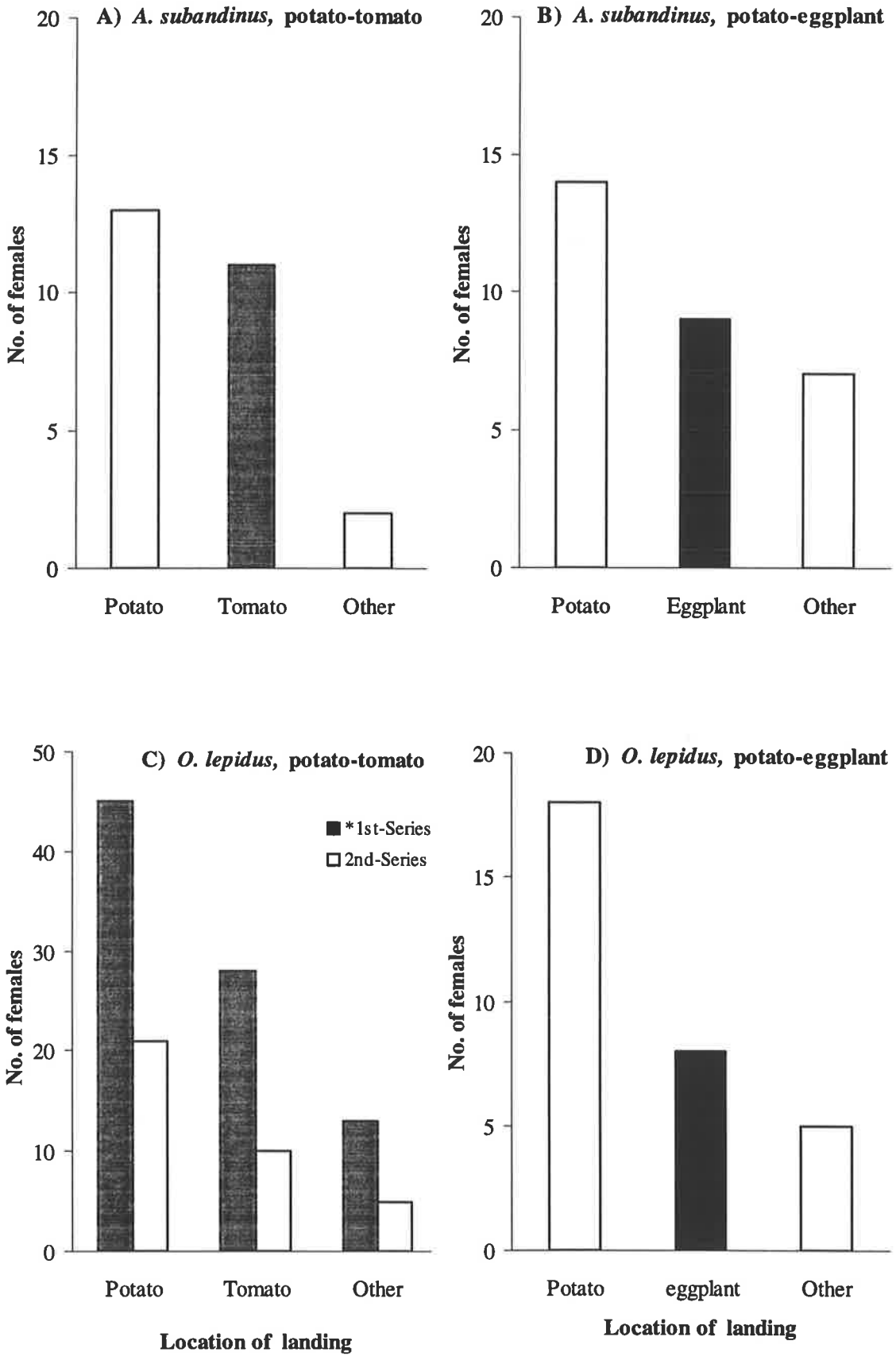
A) *A. subandinus* in choice between potato and tomato, n= 26, $P = 0.419$,

B) choice between potato and eggplant, n= 30, $P = 0.202$,

C) *O. lepidus* in choice between potato and tomato, n= 122, $P = 0.003$,

D) choice between potato and eggplant, n= 31, $P = 0.037$. The p-value based on Binomial test (Zar, 1984), using computer program written by Keller (1997).

* The first series experiments was carried out at the same time in the wind tunnel by Andries Zwaan (a student visiting from the University of Wageningen, Netherlands).


A) A. subandinus, potato-tomato
No. of females
20
15
10
5
0
Potato
Tomato
Other
B) A. subandinus, potato-eggplant
No. of females
20
15
10
5
0
Potato
Eggplant
Other
C) O. lepidus, potato-tomato
*1st-Series
2nd-Series
No. of females
50
40
30
20
10
0
Potato
Tomato
Other
Location of landing
D) O. lepidus, potato-eggplant
No. of females
20
15
10
5
0
Potato
eggplant
Other
Location of landing

infested potato and infested eggplant leaves (P= 0.202) (Fig. 5.2). Significantly more *O. lepidus* landed on infested potato than tomato (P= 0.030). In addition, a greater number of *O. lepidus* females landed on potato than on eggplant (P= 0.037).

5.4.3 Discussion

When given the choice between potato-host complex vs tomato-host or eggplant-host complex, *A. subandinus* females showed no preference to land on potato plant (P= 0.419 and P= 0.202 respectively). Regardless of cues associated with plants, *A. subandinus* females landed on each plant with PTM larvae. This suggests that *A. subandinus* would move freely between these plants when searching for hosts, but over time experience may lead them to prefer some plants over others.

O. lepidus females were more attracted to the potato-host complex than to the tomato- and eggplant-host complexes. The prior experience of the wasps on potato may have affected their choice to fly to plants infested with PTM. As have been reported in several parasitoid-host communication (see Vet and Dicke, 1992 for a review). Possibly, the complex chemical compounds from potato plant and PTM larvae are more attractive to *O. lepidus* females than chemical compounds from tomato- or eggplant-host complex. This specificity suggests that potato plant-host complex provides important cues to the searching *O. lepidus* females.

5.5 General discussion

From the results of this investigation it is concluded that *A. subandinus* females were able to distinguish from a distance of 30cm, between infested and uninfested plants with PTM larvae. The host recognition infochemicals for *A. subandinus* females are present in host plants damaged by PTM larvae. An active series of physiological and biochemical processes occurs with host feeding on plant leading to quantitative or qualitative changes in the volatiles

emitted (e.g. Du *et al.*, 1996). Odours originating from infested plant foliage appear to be important attractive stimuli for female *A. subandinus*. When infested potatoes and mechanically damaged potato plants were introduced, *A. subandinus* females were attracted to the infested potato plant, and when infested potato with infested tomato or eggplant were introduced, females were equally likely to land on either infested plant.

The attraction of *A. subandinus* to plants infested with PTM show as that females discriminated between the volatiles of mechanically damaged plants and those of host damaged plants as previously has been demonstrated for natural enemies (Vet and Dicke, 1992; Turlings *et al.*, 1990; Dicke, 1994) the weak responses observed to mechanically damaged plants show that chemicals emitted from host plant sources alone are not efficient cues for attraction of this wasp.

O. lepidus females were more attracted to potato plants with active PTM larvae than tomato and eggplant. Female wasps preferred infested potato plants with PTM larvae over uninfested plants or those with mechanical damage and its importance has already been well documented (Keller and Horne, 1993). It is not clear whether the prior rearing and experience on potato infested with hosts simply enhanced the response of *O. lepidus* to hosts on potato plants or not. The greater numbers of *O. lepidus* orientated to infested potato versus infested tomato or eggplant foliage implies that odours released from host-potato plant complex had a more attractive blend of volatiles attracted *O. lepidus* females.

The results suggested host finding by both parasitoids is stimulated by a combination of chemicals. More information is needed about the types of stimuli which attract the wasps and how their experience influences responses to these stimuli. In addition, to isolation and identification of the volatile chemicals released from the host-plant complex additional experiments are necessary to elucidate (1) sources of attractive chemicals, (2) responses to

them by both flying and walking wasps, and (3) the effects of experiences during development and adult life. Such research could shed light on how foraging behaviour influences the searching efficiency of the parasitoids. However, this area was not the primary focus of this thesis so further experimentation in this area was not pursued.

Chapter Six

The responses of *O. lepidus* and *A. subandinus* to local variation in the density of PTM

"The theory of Nicholson (1933) and Nicholson and Bailey (1935) assumes that the searching efficiency of a parasitoid as measured by its "area of discovery" is a constant and thus independent of both host and parasitoid density"

M. P. Hassell, 1985

6.1 Introduction

Knowledge of the relationship between host density and rate of parasitism is important when evaluating the efficacy of a parasitoid as a biological control agent (Huffaker *et al.*, 1971; Van Lenteren and Bakker, 1976). [IC1]One of the characters suggested by Hassell and Rogers (1972) for an ideal parasitoid in biological control is attraction to areas of high host density, or aggregation (see Murdoch *et al.*, 1987). Hassell and Rogers (1972) reported that some parasitoids and predators aggregate on certain patches with high host/prey density. Researchers of parasitoid density-dependence described two types of aggregation, aggregation in patches according to host density, and aggregation in patches independent of the host density (Godfray and Pacala, 1992). Studies in laboratory systems tend to confirm that parasitoids aggregate and spend more time in areas of high host density (Van Lenteren and Bakker, 1978; Waage, 1979), though Waage (1983) pointed out that it is rarely shown that they spend more searching time at higher densities. In addition, aggregation of a parasitoid does not necessarily imply density dependence of the rate of parasitism (Morrison and Lewis, 1984; Arpaia *et al.*, 1997). Few studies on parasitoid density dependence have been conducted in the field (Waage, 1983; Smith and Maelzer, 1986; Walde and Murdoch, 1988).

[IC2]Parasitoids increase or decrease their oviposition rate depending on host abundance, and parasitoids can aggregate or disperse according to whether the host density is high or low. In this manner, the searching capacity of a parasitoid is an important characteristic (DeBach and Smith, 1941; Holing, 1959; Price, 1972; Hassell and Rogers, 1972; Waage and Hassell, 1982; Li and Henderson, 1993). Sometimes, there may not be any relationship between parasitoid aggregation and the percentage parasitism. For example, Smith and Maezler (1986) found low rates of parasitism of red scale by *Aphytis melinus* on fruit independent of host density, despite some tendency of the wasp to aggregate on fruit with high scale density. That is, the relationship between parasitoid and host density was density-independent.

There has been no research on the effect of density of PTM larvae on the rate of parasitism by *O. lepidus* and *A. subandinus*. In this study, the influence of spatial variation of host density on parasitism by the two parasitoids was studied in the laboratory and in the field.

6.1.1 Aim

The objective of these experiments was to determine whether the rate of parasitism by *O. lepidus* and *A. subandinus* females responds to local variation in host density. This aim was addressed in two experiments which were carried out in the open wind tunnel (Section 2.4) and in the field for each parasitoid.

6.2 Open Wind Tunnel (OWT) experiments

In the OWT trials, different densities were replicated at different times, and were set out in an arena with wind crossing the patches. The patches were individual plants with different densities of hosts. Female wasps could move from patch to patch, or return to the same patch again during each test. The arena was not enclosed to prevent parasitoid emigration and females were not forced to oviposit within the experimental area. The OWT mimics field conditions more closely than a cage for studying parasitoid searching among plants. In the OWT with temperature and wind speed under control, infested potato shoots with different densities of PTM were set out randomly. The average wind velocity at three heights above the soil (10, 20 and 30cm) was 22cm/s (Appendix 2.1) and the temperature 24°C. The system was turned on 1hr before starting each test to stabilise the temperature.

6.2.1 Materials and methods

O. lepidus

Five container plants (Section 2.3) were each infested with densities of 2, 4, 8, 16 and 32 newly emerged larval PTM per plant and replicated three times, hence there was a total of 15

container plants. After 24hr, the containers were randomised in a complete block design using the procedure described by Gomez and Gomez (1984). Each row of five plants along the length of the wind tunnel formed a block (Fig. 6.1.A). Containers of plants were placed in holes in the base of the tunnel so the soil surface was flush with the bottom of the tunnel. Sand was spread over the tunnel floor. All replicates used the same lay out.

All *O. lepidus* females released in the OWT were reared on larval PTM fed on potato tubers. The selected females were fed honey and water and kept with a male after emergence. In each trial, four females 2- to 3-days-old were held in a releasing cage where they were provided with a freshly infested potato leaf with 7-10 established PTM larvae. They were given 30min experience with PTM larvae on leaves before starting each test. Such females contained on average (±sem) 144 (±13.2) eggs within their oviducts (Appendix 4.1). The releasing cage was placed in the middle of the patches and wasps were released by removing the cover of the cage. This experiment was repeated a total of 9 times under the same conditions. Exposure time was varied. The exposure of 4hr was replicated three times, and 6hr, 8hr and 10hr were replicated two times. Exposure time was varied to find changes in density response of parasitoids by increasing exposure time, and also to estimate the oviposition rate of parasitoids when the number of unparasitised hosts decreased as a result of increasing exposure time.

At the end of each experiment, the infested plants were collected and placed in the culture room for 24hr. The larvae collected from each plant were dissected using the procedure described in Section 4.3.1. The total number of hosts parasitised, superparasitised and unparasitised was recorded in each patch.

Figure 6-1: Experimental set-up with 15 potato plants infested with different numbers of PTM larvae. Numbers indicate densities in each design. Slots (7cm in diameter) are cut 15cm apart in three rows on the wooden board (64 × 140cm).

A. subandinus

Two sets of experiments with different host density distributions were carried out using *A. subandinus* in the OWT. The general procedures of these experiments were identical to those used for *O. lepidus.* Parasitism was estimated by rearing larvae on container plants.

Experiment-1

Fifteen container plants were infested with PTM larvae at three densities as follows; 2, 4 and 8 larvae per plant, each repeated five times. After 24hr, the infested plants were set up randomly in the OWT arena with the three densities replicated in five rows (Fig. 6.1.B). Four experienced females (Section 6.2.1.1) were released from a releasing cage in each replication. The duration of wasp exposure in each experiment was 6hr. After this period, the container plants were placed in rearing boxes (Section 2.2) until adult emergence. This procedure was repeated six times.

Experiment-2

In this experiment, fifteen container plants were infested with PTM larvae at three densities: 4 (8 plants), 8 (4 plants), 16 (2 plants) and 32 (1 plant). If all larvae had established, then the same total number would have been present at each density. After 24hr, the infested plants were assigned to positions using a random number table (Fig. 6.1.C). In each test four experienced wasps were released for 6hr and this procedure was repeated six times. After exposure to wasps, the plants were placed in rearing boxes similar to the previous experiment and parasitism was estimated after adult emergence.

Data analysis

The data obtained from these experiments were analysed using logistic regression techniques. All analyses were undertaken using S-Plus for Windows V. 3.3. For logistic regression the

likelihood ratio test was used to determine whether explanatory variables were significant. In addition, for logistic regression the model dispersion parameter ϕ, (which is estimated by model residual deviance divided by the model residual degrees of freedom) was approximately one. For models which were over dispersed (dispersion parameter $\phi > 1$), the dispersion was incorporated into the testing of the significance of the explanatory variables. This is achieved by looking at the change in deviance divided by the dispersion parameter ϕ and comparing this value to a chi-squared distribution with δ degrees of freedom at the 5% level. The probability of parasitism for a particular number of hosts was estimated using the method suggested by Chambers and Hastie (1992). This method was used for all experiments described in this chapter.

6.2.2 Results

O. lepidus

The actual number of hosts established on a total of 135 plants in nine replications ranged from 1 to 26 larvae per plant. The percentage of hosts establishing decreased from low to high density. The mean number of hosts parasitised during all exposure times increased over the range of host densities (Table 6.1). For plants with less than four established hosts per plant, the proportion of plants with at least one parasitised host was slightly less than one. For plants where the number of established hosts was greater than or equal to four larvae per plant, at least one host was parasitised.

Table 6.1: The numbers of established and parasitised PTM larvae at 5 densities (n= 27/density).

Nominal density of PTM	**2**	**4**	**8**	**16**	**32**
No. established	1.6±0.5*	3.0±0.7	4.8±1.2	9.1±2.5	17.4±4.0
No. parasitised	1.1±0.7	2.3±1.0	4.0±1.6	7.6±2.7	13.7±5.2

* Numbers in all data present ±sd.

Logistic regression indicated no relationship between percent parasitism and the host density (Fig. 6.2.A). Females visited virtually all plants during the different exposure times, but they oviposited in a higher number of established PTM at high densities than low densities (Fig. 6.2.B). The results of the regression analysis for all replications indicated a highly significant regression of number of parasitised hosts vs. host densities ($P< 0.01$).

The results of different exposure times indicated that the number of hosts parasitised increased with increasing exposure time. The coefficient of determination (R^2) ranged from 0.70 at the 4hr exposure time to 0.99 at the 10hr exposure time, and the slopes of regression lines also increased with increasing exposure time; however, curves overlapped at 8hr and 10hr (Table 6.2). The data showed that longer exposure times led to a higher total number of ovipositions.

Table 6.2: Estimates of regression coefficients for the relationship between host density per plant and number of hosts parasitised by *O. lepidus* at different host densities in the OWT.

Exposure time*	Slope	Intercept	R^2
4hr	0.66	-0.066	0.72
6hr	0.74	-0.057	0.92
8hr	0.91	-0.027	0.97
10hr	0.99	+0.240	0.99

* P value for all treatments was <0.001 (Regression analysis).

However, the overall oviposition rate decreased from 4.7 hosts per hour at 4hr to 3.2 host/hr at the 10hr exposure time and the marginal oviposition rate after 4hr ranged from 1.1 to 3.5 host/hr (Table 6.4). These data confirmed previous results on diminishing returns from searching when hosts are depleted (Section 4.2.2).

Larval dissection indicated that superparasitism occurred in all replicates. The number of

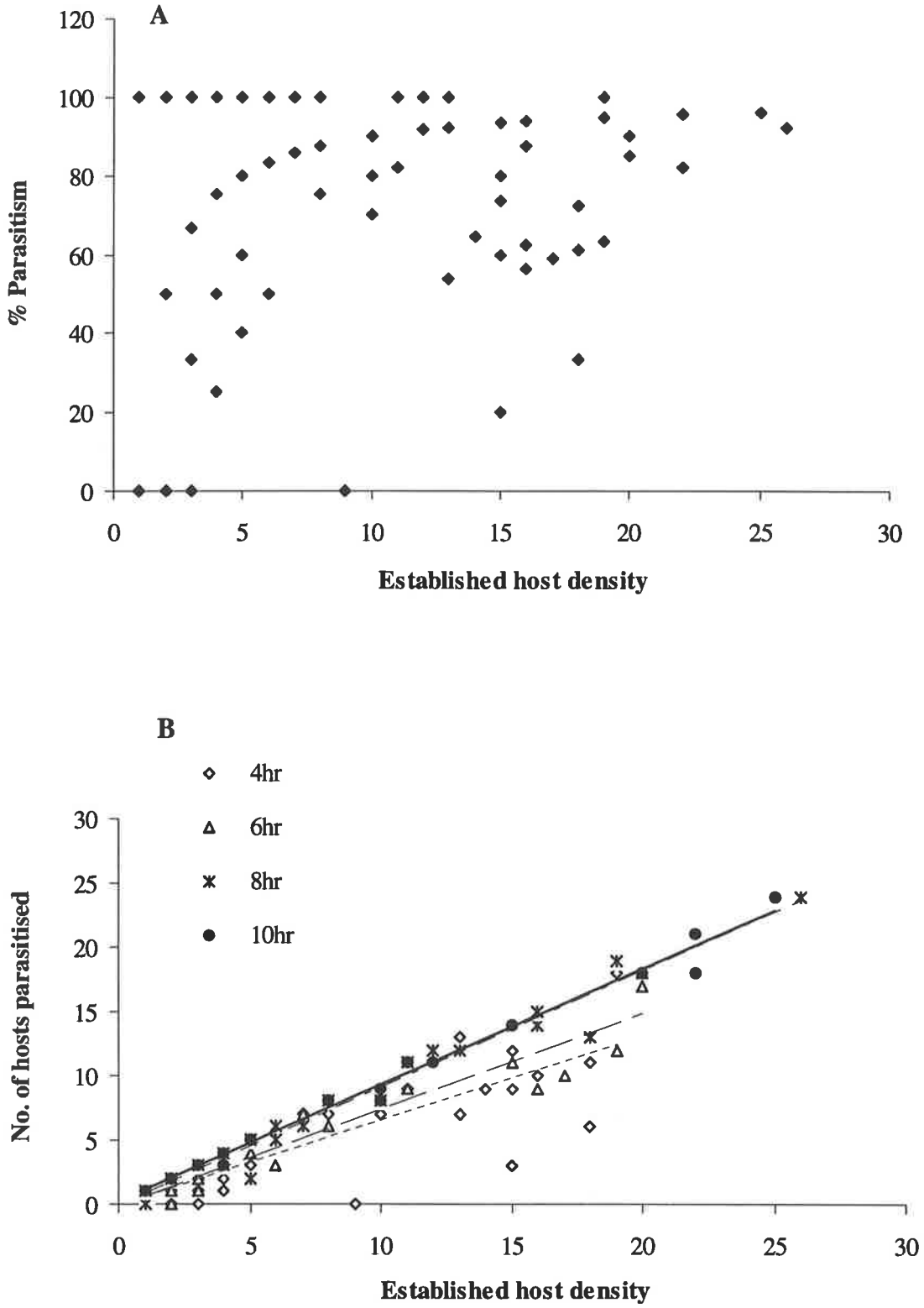


Figure 6-2: **A)** The percent parasitised hosts, and **B)** the number of hosts parasitised by *O. lepidus* at different host densities over four exposure times in the OWT. Plotted lines show linear regressions for the four exposure times. Statistical details are given in Table 6.2.

superparasitised hosts increased with exposure time (Fig. 6.3.A). The data showed that more hosts were superparasitised in patches with high host densities (Fig. 6.3.B). However, the slopes of regression lines were similar after 4hr exposure time (Table 6.3). This result confirmed that wasps were searching longer on plants with high host densities, but density-dependent parasitism was not detected.

Table 6.3: Estimates of regression coefficients of the number of superparasitised hosts vs. host density (no./plant) by *O. lepidus* at different exposure times in the OWT (see Fig 6.3.B).

Exposure time	Slope	Intercept	R^2
4hr	0.15	0.169	0.78
6hr	0.24	0.106	0.83
8hr	0.23	1.373	0.55
10hr	0.26	0.942	0.78

Table 6.4: Influence of exposure time on oviposition rate of *O. lepidus* and the corresponding marginal oviposition rates associated with each period.

Exposure time	Oviposition rate (eggs/wasp/hr)	Exposure period	Marginal oviposition rate
4hr	4.7	0 - 4hr	4.7
6hr	3.5	4 - 6hr	1.1
8hr	3.5	6 - 8hr	3.5
10hr	3.2	8 - 10hr	2.0

A. subandinus

Experiment-1

The actual number of established hosts in all tests ranged from 1 to 7 per plant. Regression analysis for all replications indicated a highly significant regression between the number of

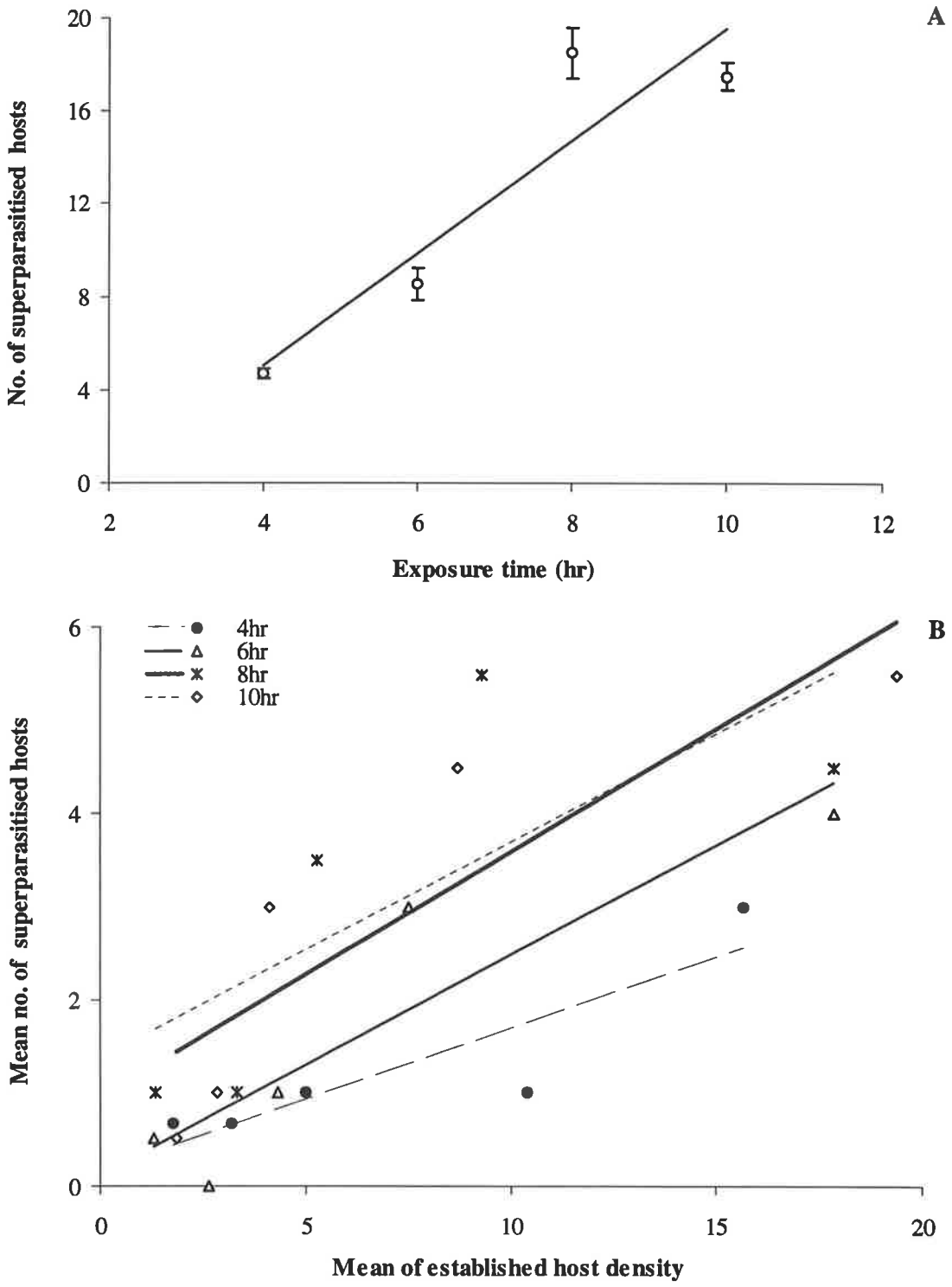


Figure 6-3: **A)** The relationship between mean number (±sem) of superparasitised hosts and four different periods of exposure when *O. lepidus* was released in the OWT. **B)** Superparasitism increased with host density. Plotted lines show linear regressions of four replicates at four exposure times. Statistical details given in Table 6.3.

hosts parasitised and host density ($P< 0.01$). A pooling of plants with the same numbers of hosts was cross-tabulated with the number of parasitised hosts per plant (Table 6.5). The data indicated that the number of plants with parasitised hosts increased as the number of hosts increased.

Table 6.5: Actual number of hosts and the number parasitised by *A. subandinus* on 90 plants infested with PTM during six replications in the OWT.

No. of hosts parasitised / No. of hosts	0	1	2	3	4	5	6	7	Total plants
1	6	3							9
2	14	10	9						33
3	2	4	6	1					13
4	3	0	3	2	5				13
5	0	1	0	1	3	2			7
6	0	0	1	1	3	1	0		6
7	0	0	0	1	1	3	2	2	9
Total	25	18	19	6	12	6	2	2	90

The final model fitted to the data was: $p = \frac{\exp(-0.8197 + 0.2832(No.of.Hosts))}{1+\exp(-0.8197 + 0.2832(No.of.Hosts))}$, where p is the probability of parasitism. This model was found to be slightly over-dispersed with an estimated dispersion parameter ϕ of 1.9. After taking into consideration the higher than expected dispersion parameter the number of hosts was found to significantly influence the probability of parasitism at the 5% level. The results of the logistic regression model showed that the probability of an individual host being parasitised increased as the number of hosts increased (Fig. 6.4).

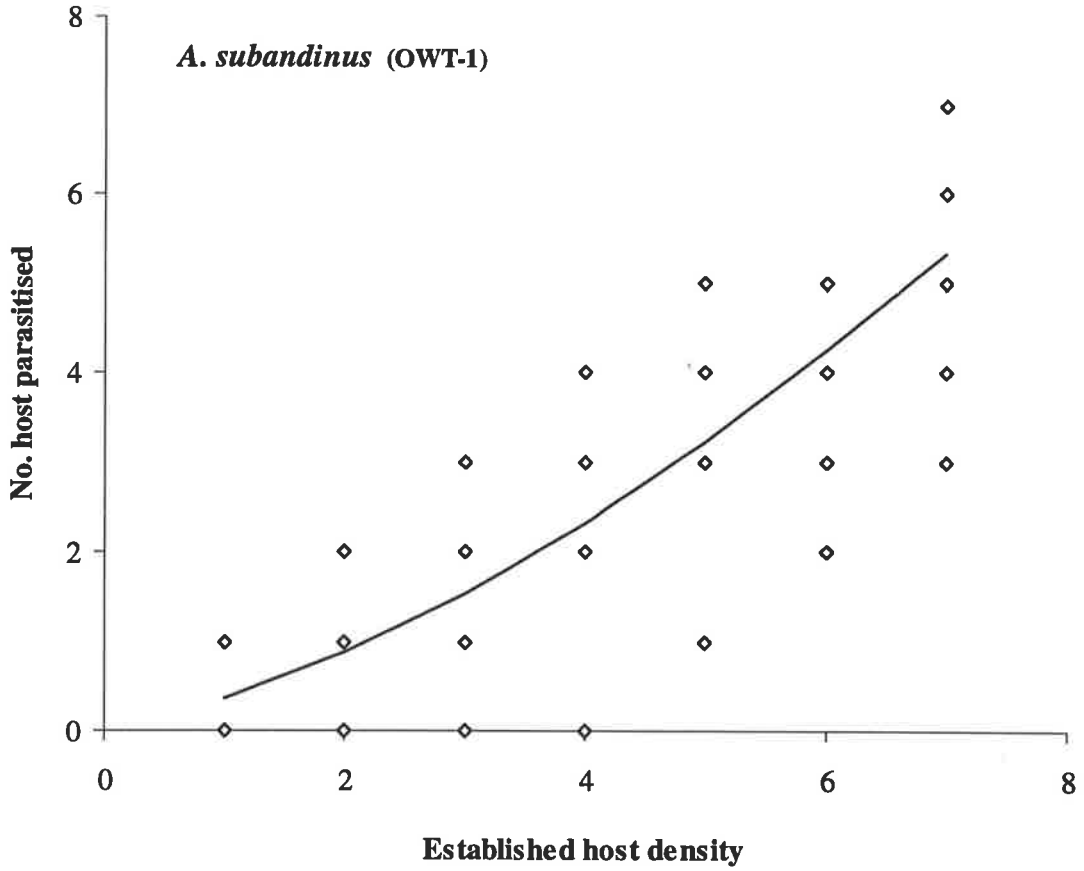


Figure 6-4: Parasitism by *A. subandinus* when 2, 4 and 8 larvae were released on each plant and each initial density was replicated 5 times. The line shows the probability of parasitism and the expected mean number of hosts parasitised for each host density.

Experiment-2

The actual number of established hosts ranged from 2 to 22 per plant in this experiment. Regression analysis indicated that the number of hosts parasitised increased with increasing host density (Fig. 6.5.A).

There was large variation in the level of host establishment of each group, with many plants having less than five established larvae. Initial analysis suggested that the proportion of parasitised hosts did not show significant differences according to variation in host density. However, all plants had at least one host parasitised when host density exceeded 10/plant (Fig. 6.5.B). The number of plants with less than five larvae was higher than plants with more than five larvae. A refined logistic regression model indicated that the number of hosts present on plants affected the probability of parasitism. The number of hosts was fitted as a categorical variable with two levels (≤4, or >4)and the replicate number was fitted as a categorical variable with six levels.

The final model fitted was of the following form: $\log(\frac{P}{1-P}) = \mu + h_i + r_j$ where P is the probability of parasitism, 1-P is the probability of not being parasitised, h_i = host number categorised into two levels {i =1 (number of hosts≤4 and i =2 (number of host>4)} and r_j = replicate number categorised into 6 levels. The model presented the log odds ratio of parasitism as a function of the level of host number. This model indicated that if the number of hosts was greater than four larvae per plant, the chance of parasitism on that plant improved by a factor of 2.2177 (exp(0.7965)) compared to when the number of hosts was less than or equal to four larvae per plant. The 95% confidence interval for this factor is 1.3437 to 3.6604.

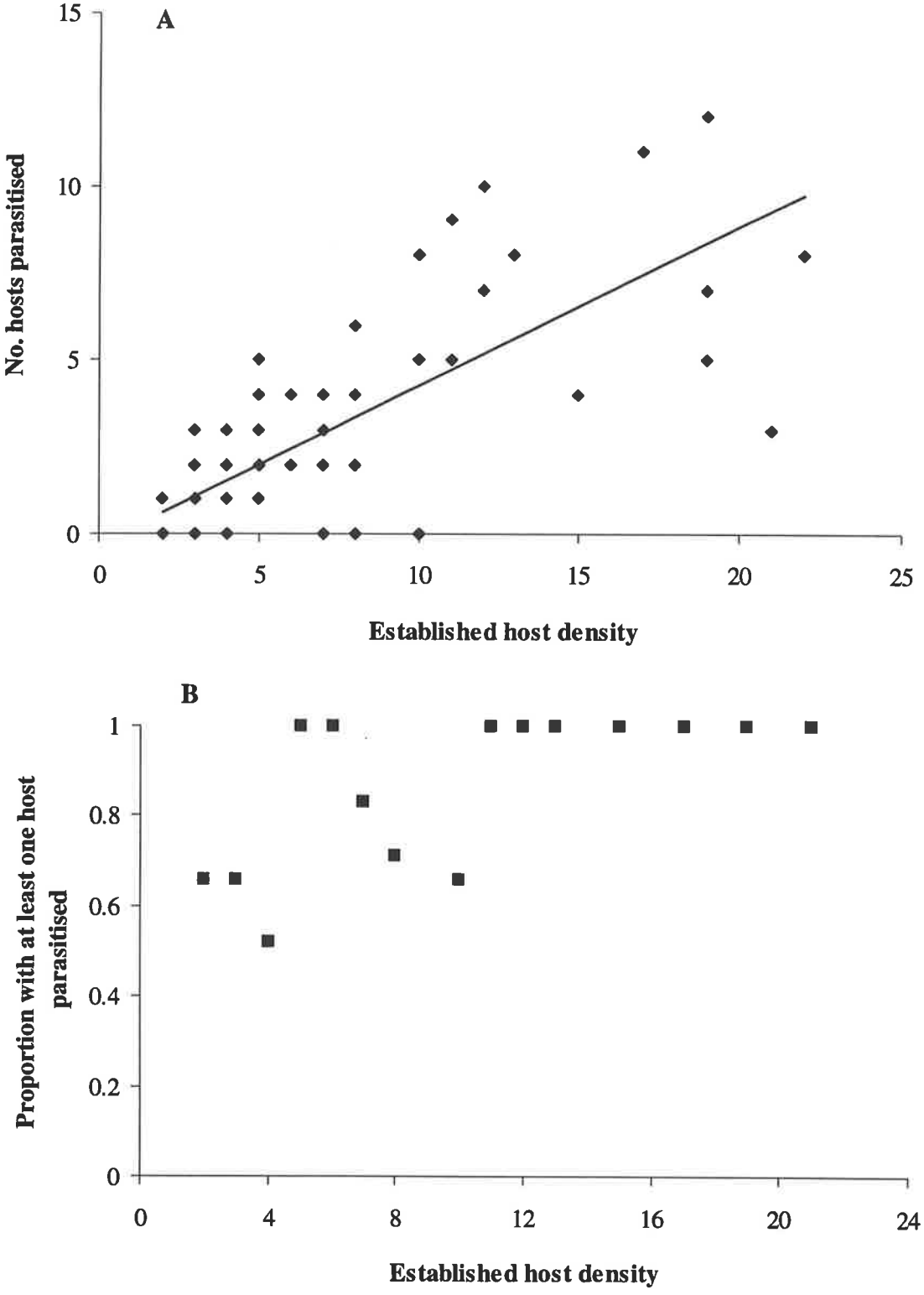


Figure 6-5: **A)** Parasitism by *A. subandinus* when the numbers of larvae released onto plants were 4 (8 plants), 8 (4 plants), 16 (2 plants) and 32 (1 plant), line indicates linear regression of all replicates, and **B)** proportion of plants with at least one host parasitised, at the different host densities.

6.3 Field experiments

The field experiment was carried out using potted potatoes infested with newly emerged PTM larvae at different densities at the Waite Campus, The University of Adelaide. This area was free of both Solanaceous crops and the insects under investigation.

6.3.1 Materials and methods

O. lepidus

The response of female *O. lepidus* to local variation in host density was studied over four host densities replicated three times, constituting one experiment. This one experiment was repeated four times and the procedures of each experiment were similar except in the number of larvae per plant which doubled in the third and fourth experiments. Temperature varied between 22 and 30°C during the experiments. Potatoes (of the var. Colliban) were grown in plastic trays (42 × 32 × 12cm) to a height of 30 to 35cm in a glasshouse. Twelve trays with similarly sized plants were infested with 1-day-old PTM larvae at densities of 10, 20, 40 and 80 PTM larvae per tray. The infested plants were transferred into the field after 24hr and placed around a circle 2 meters in diameter (Fig. 6.6.A). Fourteen 2-3-day-old females of *O. lepidus* were placed in a releasing cage with an infested potato shoot (Fig. 2.5) and placed in the centre of the circle 1hr before starting the experiment. Then the wasps were released and allowed to search for 7hr. At the end of the experiment, the foliage from each tray was placed in rearing boxes and held for adult emergence.

Because of the thick dense foliage in each tray, host densities were increased to 20, 40, 80 and 160 larvae per tray plant in the third and fourth experiments. The number of females released was decreased to 10 wasps and exposure time was 6hr.

Figure 6.6: Design of the field experiments, plants in trays with four host densities replicated 3 times for *O. lepidus* (**A**), potted potatoes with four densities replicated four times for *A. subandinus* (**B**).

A. subandinus

Sixteen potted potato plants 30 to 35cm high were infested at densities of 5, 10, 20 and 40 larvae per plant. These plants were also transferred into the field after 24hr and set out randomly around a circle 2 meters in diameter (Fig. 6.6.B). Ten 2-3-day-old female *A. subandinus* were released using the same methods as were used with *O. lepidus*. After six hours, the plants were collected and larvae reared as was described for *O. lepidus*.

Data analysis

Data obtained from experiments in the field were analysed using a logistic regression model using S-Plus. The probability of parasitism was estimated with the method suggested by Chambers and Hastie (1992) (see Section 6.2.1).

6.3.2 Results

O. lepidus

The actual numbers of hosts established in the trays of plants ranged from 5 to 53 in replicates 1 and 2 and from 13 to 100 larvae per tray in replicates 3 and 4. The mean ±sd of parasitism varied from 40.6±13.0% to 70.1±4.7% per tray in replicates 1 and 2, and ranged from 13.8±7.8% to 43.4±8.5% in replicates 3 and 4 for the lowest to highest densities respectively (Table 6.6 all plants except one with 14 established larvae had at least one host parasitised).

The final logistic regression model indicated that the number of hosts and the replicate number significantly affected the probability of parasitism (Fig. 6.7.A). In addition, there was an interaction between the number of hosts and the number of replicate, therefore, the relationship between parasitism and host number was examined separately for each replicate.

Table 6.6: The number of established PTM larvae per plant at 4 densities in 2 of sets of experiments in the field.

Experiment	Density of PTM	No. larval PTM released				
		10	**20**	**40**	**80**	**160**
1 and 2	Mean established	6.0±0.9*	13.7±1.2	21.3±1.5	46.7±5.6	-
1 and 2	Mean %parasitism	40.6±13.0	58.4±9.0	63.4±8.5	70.1±4.7	-
3 and 4	Mean established	-	14.7±2.3	25.2±4.7	51.0±4.2	94±4.0
3 and 4	Mean %parasitism	-	13.8±7.8	36.9±9.5	32.5±17.3	43.4±8.5

* Numbers in all data present ±sd.

The final models fitted for replicates 1 and 2 and replicates 3 and 4 were:

$$\log(\frac{P}{1-P}) = 0.04389+0.01734 \text{ (Number of hosts)} \qquad \text{Replicates 1 and 2}$$

$$\log(\frac{P}{1-P}) = -1.4779 +0.04832 \text{ (Number of hosts)} \qquad \text{Replicates 3 and 4}$$

Where P is the probability of parasitism and $(1-P)$ is the probability of not being parasitised.

For replicates 1 and 2 an increase of one host on a particular tray of plants improved the chance of parasitism of hosts multiplicatively by a factor of 1.0175(=exp(0.01734)) and for replicates 3 and 4, it was 1.0495(=exp(0.04832)). This factor lies within the 95% confidence interval 1.0042 to 1.0310 for replicates 1 and 2 and 1.0369 to 1.0623 for replicates 3 and 4.

A. subandinus

The number of established hosts ranged from 2 to 28, and plants with the same number of larvae were combined and compared with other densities. The mean number (±sd) of hosts established on a total of 48 potted potatoes in three replicates ranged from 3.3 (±0.8) to 18.9

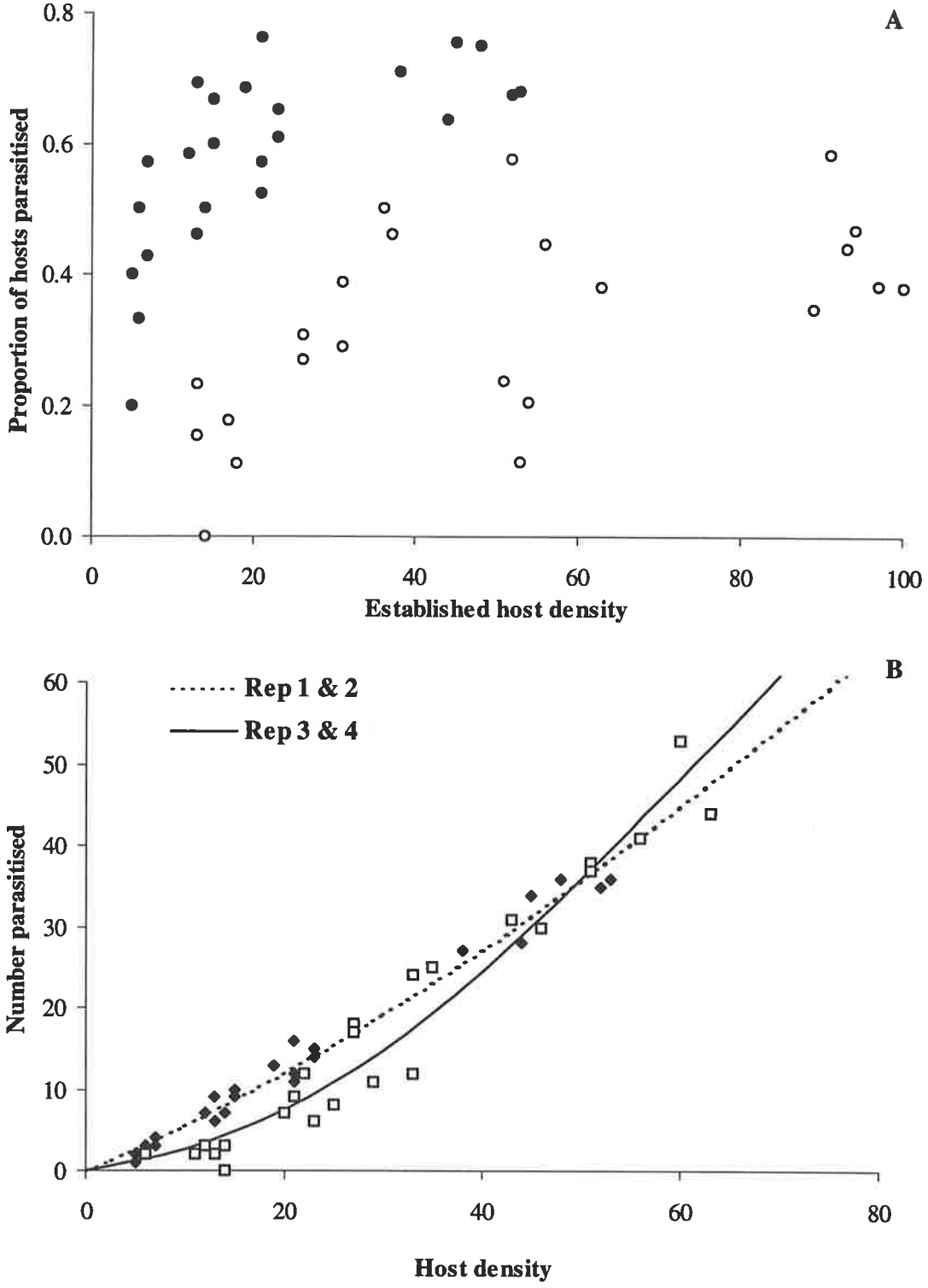


Figure 6-7: **A)** The proportion of parasitism by *O. lepidus* on different host densities in the field, solid circles represent replicates 1&2 and empty circles represent replicates 3&4, **B)** the number of hosts parasitised, the predicted lines are estimated from final models (Section 6.3.2) for replicates 1&2 (broken line) and replicates 3&4 (solid line).

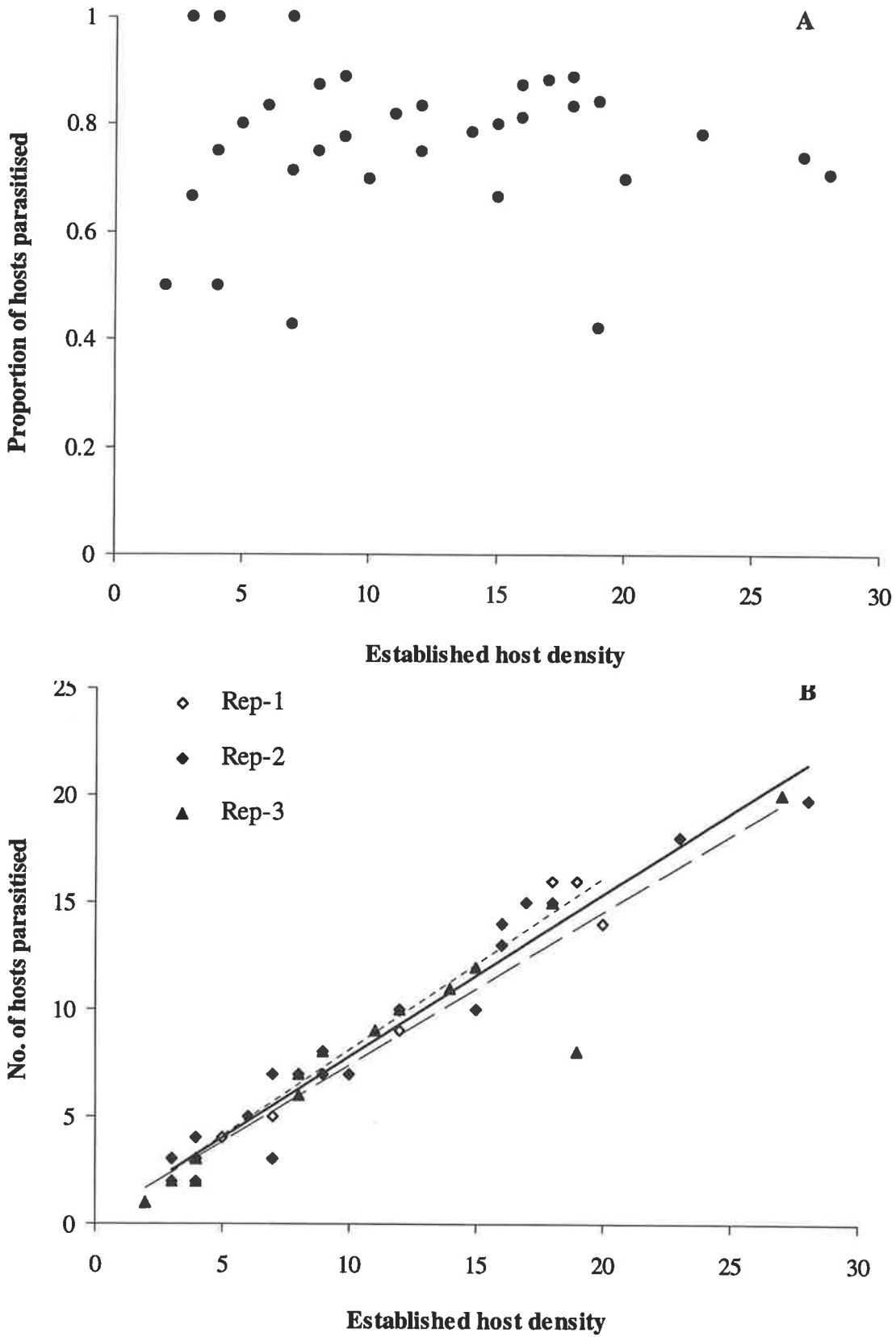


Figure 6-8: **A)** The number of hosts parasitised by *A. subandinus* in the three replicates in the field, **B)** the rate of parasitism was very close in three replicates as shown by the R^2 (0.97, 0.95, 0.85) and slopes (0.80, 0.75, 0.71) for replicates 1,2 and 3 respectively.

(±6.1) larvae per plant and the mean number of parasitised hosts varied from 2.5±0.9 to 14.3±5.9 larvae at the lowest and highest host densities. On all plants there was at least one host parasitised. The proportion of hosts parasitised at different densities did not vary with host density (Fig. 6.8). These results indicated that female *A. subandinus* searched all plants and oviposition increased in proportion to the numbers of hosts available on plants, but density-dependent parasitism was not observed.

6.4 General discussion

The results of laboratory and field experiments indicated that the two parasitoids differed in their responses to local variation in host density. Their tendency of aggregation and oviposition in high host density patches varied with changes in conditions, such as the range of host densities, plant size and available searching time in the laboratory and field experiments.

The mean percent parasitism of *O. lepidus* displayed a density independent response in the experimental design used in the laboratory. This design involved a relatively artificial spatial arrangement of small plants with host density ranging from 1-26 larvae per plant. The percent parasitism by female *O. lepidus* was very high with no difference in low and high host densities in the OWT. In contrast, the results of field experiments indicated that there was a significant positive relationship between percent parasitism and host density by *O. lepidus*.

Direct observations indicated that female *O. lepidus* aggregated and oviposited on plants with high host densities. Females that landed on a plant with a low density soon moved to high density plants. They did not search for long on plants with very dense foliage and low numbers of hosts, but they searched longer on similar plants with high host densities. Like many parasitoids, it is possible that *O. lepidus* females are directed to their host through a series of chemical cues (Vinson, 1976) and these cues elicit a series of direct responses by the

female to the area and habitats in which hosts are located. However, factors such as host density and kairomone concentrations could also influence the patch-leaving tendency of the wasps (Hubbard and Cook, 1978; Waage, 1979; Van Alphen and Galis, 1983).

In the OWT, parasitoids visited all plants and because plants had less foliage, they found and oviposited in a high percentage of hosts in each plant. In addition, climate was constant and food was accessible for wasps during their searching time. Furthermore, the range of host numbers per plant was small in the OWT experiments. Therefore, females *O. lepidus* searched all densities in the OWT patches. The chance of encountering an unparasitised host increased in different host densities with increasing exposure time, and the number of parasitised and superparasitised hosts also increased. However, after 8hr, parasitism was stable and there was overlap of the regression lines of 8hr and 10hr exposure time. The data obtained in this study is agreement with Hubbard and Cook (1978) finding who reported that parasitoids spend less time on patch when there are fewer unparasitised hosts present.

In the field, plants had dense foliage (approximately 20-fold larger than container plants used in the OWT experiments), climate varied on different experiment days, food was not provided for wasps during their searching time and the range of host numbers per plant was larger in the field experiments. Thus, different factors affected the changes in percent parasitism by *O. lepidus*. In contrast to the laboratory experiment, *O. lepidus* did exhibit density-dependent parasitism in the field. Perhaps this response is influenced by the wasps ability to assess host density and the frequency of parasitised hosts on patches, as well as the characteristics of the host plants.

Lessells (1985) divided the foraging time of a parasitoid into searching time, rejection time and handling time. In the OWT, the number of superparasitised hosts increased with increasing exposure time from 4hr to 8hr in all patches, because encounters with unsuitable

hosts (i.e. hosts mined inside the veins) were greater at 8hr than 4hr exposure time. Furthermore, a number of other factors have been found to contribute to, or modify, patch time and the migration from patches (see Nelson and Roitberg, 1995).

In order to understand the density-dependent responses of parasitoids it is useful to consider theories of optimal patch-leaving behaviour. In an ecological context, the optimal patch-leaving times (Godfray, 1994) have been predicted for parasitoids (Charnov, 1976; Fretwell and Lucas, 1970; Cook and Hubbard, 1977). It seems there are two factors which are relevant to a female's decision to leave a patch. Firstly, the distribution of female wasps among patches of various quality, which relates directly to the theory of ideal free distribution (IFD) (Fretwell and Lucas, 1970); secondly, the leaving tendency of individual wasps when their rate of fitness gain decreases in that patch, which relates directly to Charnov's marginal value theory (Charnov, 1976; Cook and Hubbard, 1977).

In both laboratory experiments, female *A. subandinus* showed a higher percent parasitism in high host density than low density. However, the percent parasitism was dependent on the distribution of host density.

Percent parasitism by *A. subandinus* was not significantly correlated with the density of PTM in the field experiments. It is possible that the apparent density-independence of *A. subandinus* is related to the size of plants and the number of hosts examined in these experiments. The results were similar to those reported by other researchers (e.g. Force and Moriarty, 1988; Walde and Murdoch, 1988; Rothman and Darling, 1990) who found a conditional density dependence of parasitoids. Rothman and Darling (1990) reported spatial positive density-dependence of four parasitoid species of goldenrod gall moth, *Gnorimoschema gallaesolidaginis*, when the scale of host density variation was low, and density independence for a larger spatial pattern.

The number of hosts parasitised by *A. subandinus* and *O. lepidus* on plants increased by increasing the number of hosts per plant in both laboratory and field experiments. However, female *O. lepidus* oviposited in more hosts than female *A. subandinus* during the searching period. Many investigations have found that the ability of parasitoids to respond to high host densities may be constrained evolutionarily by their rates of searching, handling and egg production (Hassell, 1982; Waage, 1983; Hassell *et al.*, 1985). It is possible that a difference in egg production between *A. subandinus* and *O. lepidus* (Section 4.3.2) is responsible for the difference in aggregation behaviour between the two species.

In conclusion, the results indicated changes in density dependence of the two species during the given time, and this was dependent on plant size and the range of host densities that were used. It appears that wasps are able to assess and respond to varying host densities under some conditions and not others.

Chapter Seven

Functional responses of *A. subandinus* and *O. lepidus*

"Functional response is response of a natural enemy to varying density of their host or prey"

M. E. Solomon, 1949

7.1 Introduction

Functional response is defined as the change in the number of hosts or prey attacked per unit of time by an individual parasitoid or predator as host or prey density changes, and is a measure of the effectiveness of the searching activity of parasitoids or predators (Solomon, 1949). The functional response of a parasitoid to its host is the result of a complex interaction involving the length of time that the host is exposed to the parasitoid, the searching behaviour of the parasitoid, the defensive behaviour of the host, the resulting proportion of successful attacks and the handling time required for each host (Holling, 1963). The response of a parasitoid or predator to host or prey density is of fundamental importance for biological control (Holling, 1959a&b). Three basic types of functional response have been defined (Section 1.3.5.3). [IC1]Functional response measurements determined in the field seem to be better than laboratory descriptions for predicting the performance of a natural enemy within a biological control program (Hughes, 1989). However, it is difficult to evaluate the efficiency of species in regulating host population from single-patch, single-parasitoid experiments (Jervis and Kidd, 1996).

This chapter was set out to measure the functional responses of *A. subandinus* and *O. lepidus* to PTM. Furthermore, the effect of host density on the proportion of female offspring of the two parasitoids was examined. These data, in combination with data from related studies in other chapters (Chapters 4 and 6), could be used to infer the effectiveness of these species at different densities of their host. Although previous chapters share similarities to the present one in terms of analysing the influence of host density on parasitism, the present chapter extends this analysis to a broader spatial scale, by using experiments in the field.

7.1.1 Aims

The hypothesis for this study was that the number of PTM larvae attacked by the parasitoids

A. subandinus and *O. lepidus* changes as host density changes. The aim of this study was to measure the number of hosts attacked by *A. subandinus* and *O. lepidus* at different densities of PTM and to determine which of the functional response models best describes their responses.

7.2 Materials and methods

Field experiments were conducted under standardised conditions in terms of host age and instar, insectary rearing conditions, host plant quality and age of wasps. Days with similar temperatures were chosen in order to limit climatic variation among replicates. The procedures for the two parasitoids were the same except that host densities per plant and the dates of experiments were different. The host densities used for *O. lepidus* were 15, 30, 60 and 120 larvae released per potted potato. Numbers of hosts were lower for *A. subandinus*, with 10, 20, 40 and 80 larvae released per plant. These densities were chosen because previous results (see Chapter 4) indicated that oviposition rates of *A. subandinus* were lower than those of *O. lepidus*.

Importantly, previous experiments (Section 6.3.2) had indicated that only 50%-60% of larvae placed on plants became successfully established. Densities higher than the above were not used because it was considered that such densities would not occur in nature. Indeed, the highest densities used are probably substantially greater than those which typically occur in the field.

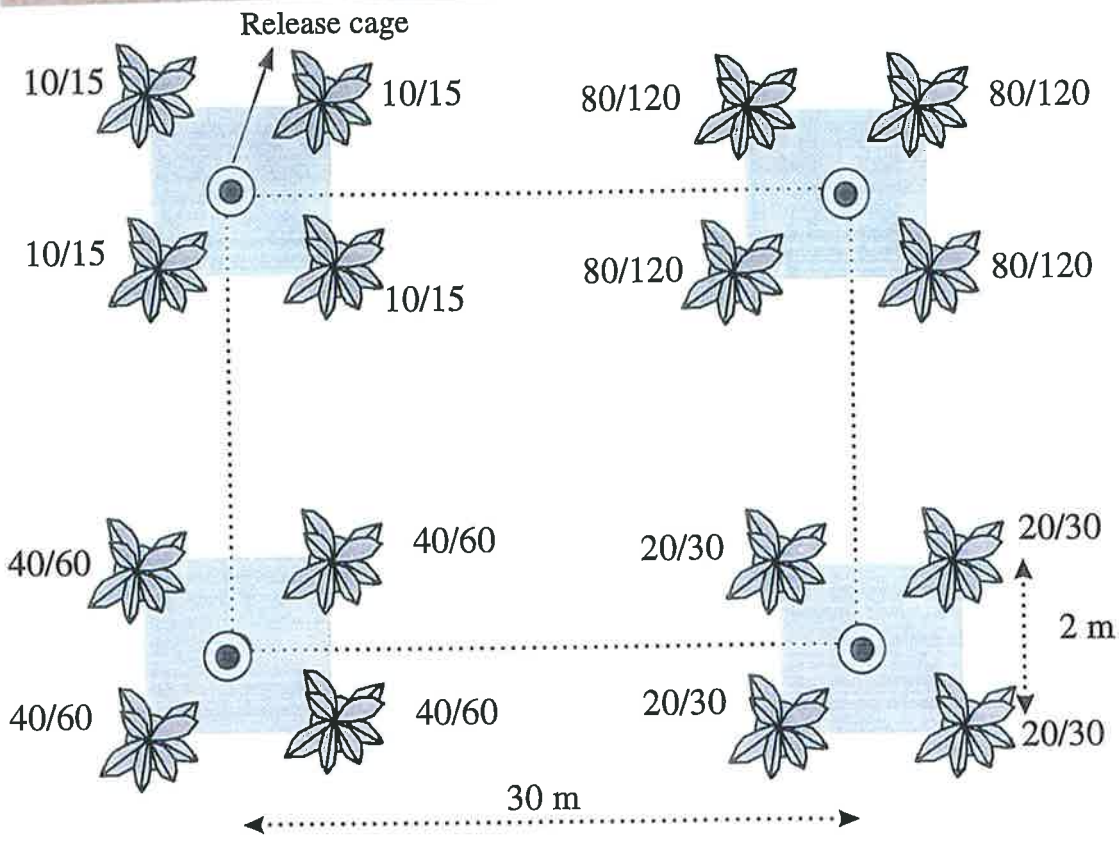
Four replicate pots of potatoes per density were used in each experiment. These potted plants were infested with the same initial numbers of hosts and were set out at the corners of a square measuring 2m × 2m. These four plants were used to represent a foraging patch. Four such patches, each with a different host density, were arranged at the four corners of a larger square measuring 30m × 30m (Fig. 7.1). Four gravid female wasps were released in the centre

Figure 7.1: Design of the functional response experiments in the field:

A) Photograph of the four patches each containing four potted potato infested with the same number of PTM larvae,

B) One patch with releasing cage in the centre,

C) Diagram to show experimental design for *A. subandinus* with host densities of 10, 20, 40, 80 larvae per plant and for *O. lepidus* with host densities of 15, 30, 60, 120 larvae per plant.


Release cage
10/15
10/15
10/15
10/15
80/120
80/120
80/120
80/120
40/60
40/60
40/60
40/60
20/30
20/30
20/30
20/30
2 m
30 m

of each of the smaller squares. These wasps were 2-3-days-old to avoid the possibility that low egg loads would influence their foraging behaviour, as might be the case for older wasps. Egg load has been reported as an important factor influencing foraging behaviour of parasitoids (see Minkenberg *et al.*, 1992). The 2-3-day-old wasps used in this experiment have predicted mean (±sem) egg loads of 144 (±13.2) for *O. lepidus* and 121 (±13.0) for *A. subandinus* (Section 4.3.2). These egg loads were considered high enough not to be limiting in this experiment.

To determine if wasps moved between patches in the field experiment, the wasps were marked on the thorax with coloured enamel paint. Wasps were painted using a fine brush (one hair). Four colours (yellow, blue, red and white) were used; one colour for each of the four foraging patches.

All experiments were set up in the morning and the wasps allowed to search for 4hr. Hourly observations of the patches were made to record the movement of parasitoids. At the end of the experiment each pot plant was thoroughly checked to find and remove wasps, then placed in a cage (size 33cm × 26cm × 26cm) and transferred to the insectary. Infested foliage collected from each potted potato was placed in a rearing box and provided with tubers and white sand.

Analysis

In the data analysis, the actual density of larvae established was used instead of the initial number of hosts placed on plants, because not all the larvae established on the plants. The data used were the means for each foraging patch, i.e. the mean initial host density and mean number of hosts parasitised, averaged over all four plants within each foraging patch. To help assess the impact of variation in temperature between replications on different days of each experiment, the temperature during wasp release hours was obtained from the Waite

Meteorological Station at The University of Adelaide (approximately 300 meters from the study site).

These experiments were repeated six times for *O. lepidus* and five times for *A. subandinus*. Data analysis of the functional response experiments was conducted by fitting linear and non-linear regression models (see Section 1.4.3.5 for model equations) of Type 1, 2 and 3 functional responses (Holling, 1959a & b, 1961) using the GLM and NLIN procedures of SAS (SAS Institute, 1995). The adjusted coefficient of determination for the relationship between host density and rate of parasitism by the two parasitoids was estimated using Kvalseth's (1985) equation.

7.3 Results

For *O. lepidus* the mean number of established hosts (±sd) varied from 9.7 (±1.0) larvae per plant on low density plants to 63.5 (±) 16.0 larvae per plant on high density plants. The mean number of established hosts for *A. subandinus* experiments varied from 5.9 (±1.6) larvae per plant and 30.5 (±11.4) larvae per plant.

O. lepidus

The number of hosts parasitised by *O. lepidus* increased as host density increased. While a Type 2 functional response model provided the best fit for the data, the fit was only marginally better than that for the other two models (Table 7.1). Importantly, there was no clear evidence of a plateau in the rate of parasitism at higher host densities (Fig. 7.2). Although temperature varied between the six replicates (ranging from 21 to 28°C), it did not show significant effects on the rate of attack in the field.

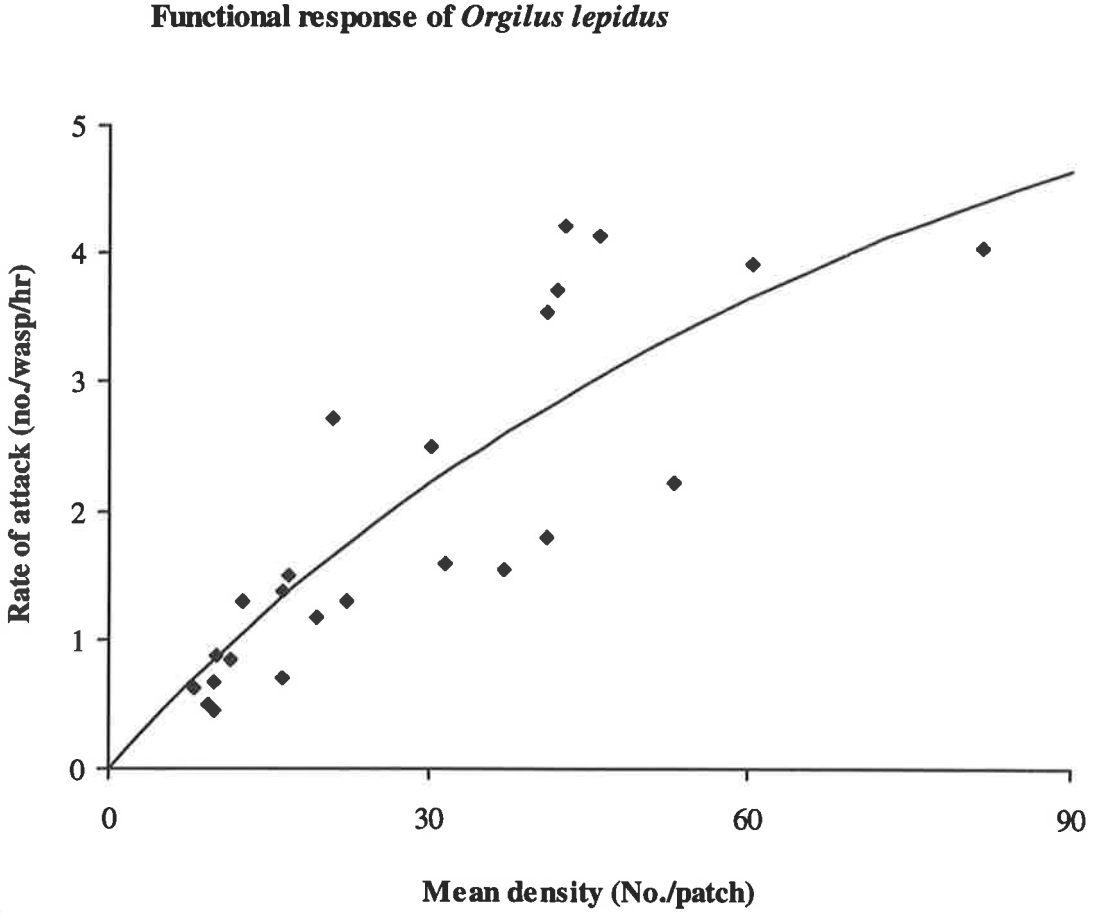


Figure 7.2: Functional response of *O. lepidus* to PTM density. Symbol represents numbers of parasitised hosts over the four patches for six replications. Line indicates the curve predicted by the Holling Type 2 model (N =24, $a = 0.093, T_t = 1$, $h_t = 0.096$).

A. subandinus

The number of hosts parasitised by *A. subandinus* increased with host density increased. The adjusted coefficient of determination for the relationship between host density and rate of parasitism was used to choose the best curve for the data with and without the effects of temperature (Table 7.1). A Type 2 functional response model provided a better description of the data than the Type 1 or Type 3 models if the effect of temperature on the rate of attack by wasps is ignored. The mean daily temperature for each of the five replicates did vary somewhat, ranging from 18.0°C to 29.0°C. The number of hosts parasitised in all patches on the day with average temperature 29.0°C was higher than the others (Replicate 4 in Fig. 7.3). A Type 1 functional response model provided the best description for the data when temperature was included as a factor.

Table 7.1: Comparison of the adjusted coefficients of determination Kvalseth, 1985) which indicate the best fit of four functional response models to the observed behaviour of *O. lepidus* and *A. subandinus.*

Parasitoid	Type 1	Type 1 with Temp.	Type 2	Type 3
O. lepidus	0.655	-[†]	0.699	0.693
A. subandinus	0.225	0.490	0.326	0.301

[†] Temperature did not have a statistically significant effect on the predicted rate of parasitisation.

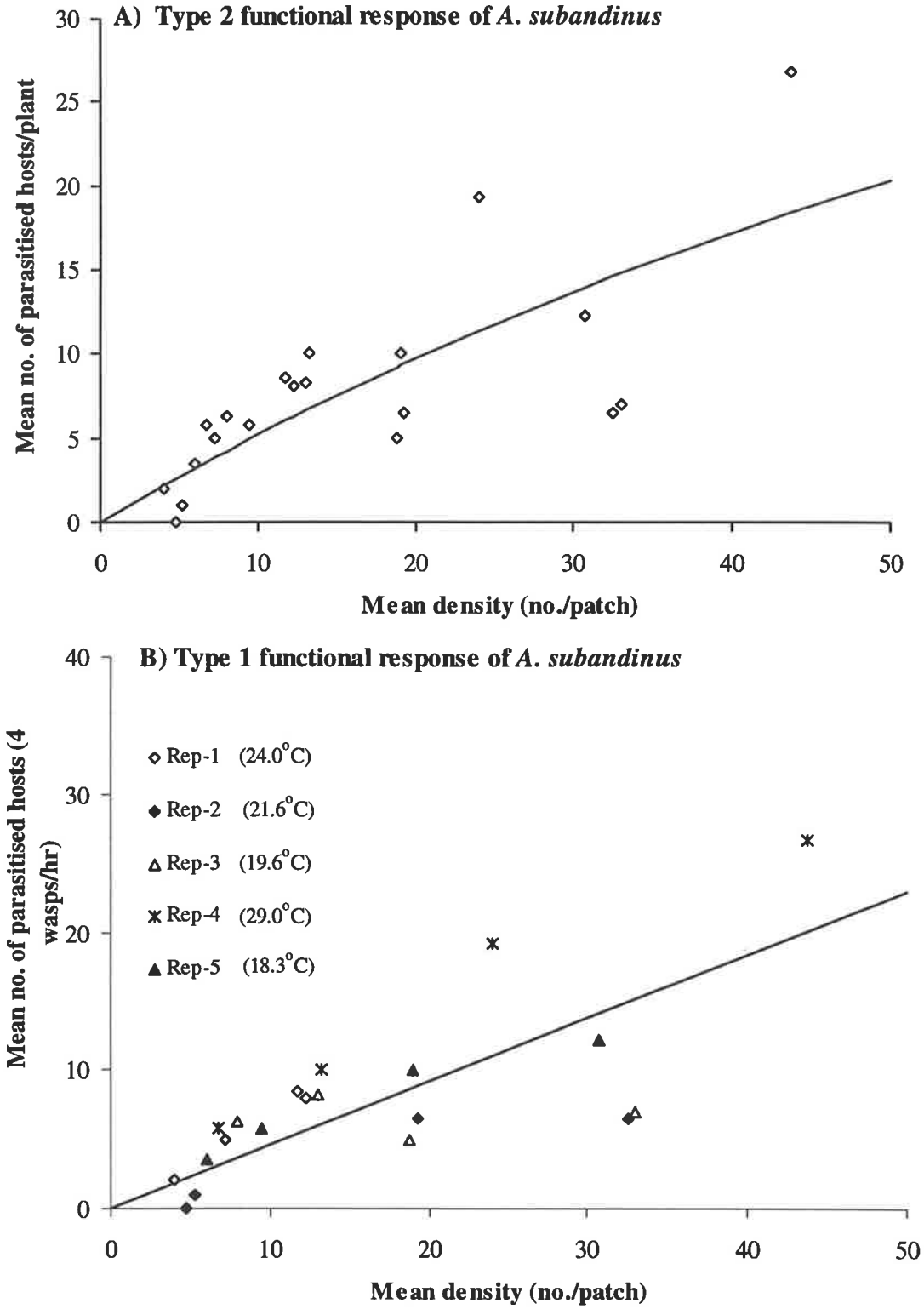


Figure 7.3: Functional response of *A. subandinus* to PTM density. **A)** Holling Type 2 model is indicated ignoring the effect of temperature ($N = 20$, $a = 0.15, T_t = 1$, $h_t = 0.28$). **B)** Holling Type 1 model is indicated with temperature, the line represents the predicted functional response at the mean temperature of 22.5°C ($N = 20$, $a = 0.003, T_t = 1$).

Inter patch movement

Inspection of all plants showed no evidence of inter patch movement by marked individuals of either species during the experiments. However, in all patches and particularly at low host densities, fewer than four wasps were recovered at the end of each 4hr experimental period. While the fate of these wasps is unknown, it seems likely that they had left patches in search of other hosts.

7.4 Discussion

Quite clearly, the mean number of hosts parasitised by both *O. lepidus* and *A. subandinus* increased with increasing host density. The previous results in Chapters 4 and 6 indicated that oviposition by the two parasitoids was directly influenced by host density and that the proportion of oviposition was greater at high host density than at low density.

An increase in the rate of parasitism with host density could be important in helping to stabilise a parasitoid-host interaction (Hassell and Rogers 1972). The increase in the rate of parasitism with host density was found to be greater in *O. lepidus* than *A. subandinus* (Chapters 4 and 6). This could be because *O. lepidus* has a greater egg load than *A. subandinus* and can therefore oviposit in a greater number of hosts over a given time period (Section 4.3.2). Furthermore, it seemed that when pots were collected from the field at the end of each exposure time for *O. lepidus*, a greater number of *O. lepidus* were searching on high density patches than on low density patches.

A Type 2 functional response provided a good description of the increase in the rate of parasitism by *O. lepidus* with host density, as indicated by the relatively good fit of this model to the data. However, the suitability of a Type 2 model over Type 1 and 3 models could be considered marginal, given that no clear plateau was apparent. It could be agued that too small a range of host densities was used to distinguish between the three types of differences

between the three models in terms of goodness of fit. However, the highest densities used are extreme when compared to those observed in the field (Personal observations).

It seems that for *O. lepidus*, the variation in temperature between the six replicates was not an important factor influencing the shape of the functional response curves. This is perhaps not surprising, given that earlier results (Section 4.4.2) demonstrated that the parasitism rate of *O. lepidus* is relatively insensitive to changes in temperature, particularly over the range of temperatures experienced in the field experiment (21 to 28°C) compared constant temperatures in incubators (15 to 35°C).

For *A. subandinus*, a Type 2 functional response provides the best description of the data, but only if the effect of temperature is not included. When the effect of between-replicate variation in temperature was included, a Type 1 functional response curve provided a better fit for the data. Type 1 functional responses have been found for other parasitoids in field cages (e.g. Morales-Ramos and Cate, 1992; Wiedenmann and Smith, 1993). It would seem that variation in temperature between the five replicates biased the results and may have created an artificial Type 1 functional response curve. Temperature must have affected the functional response parameters {i.e. b (rate of attack) and ht (handling time)} because it affects the rate of oviposition by wasps. Importantly, previous laboratory data (Section 4.4.2) showed that the parasitism rate in *A. subandinus* is highest at approximately 30°C. It was therefore suspected that higher parasitism rates on the 29°C day may have somewhat biased the shape of the curve. Changes between the types of functional response in relation to changes in temperature have been reported in other studies (Mack and Smilowitz, 1982; Gresens and Cothran, 1982). Mack and Smilowitz, (1982) described a temperature-mediated functional response equation and found that rates of attack were linearly related to temperature. Hopper and King (1986) examined the impact of air temperature on searching rate of parasitoid *Microplitis croceipes* and found that search rate increased linearly with temperature. Van Roermund *et al.* (1994)

reported that temperature influenced the walking speed of parasitoid *Encarsia formosa*. They found that temperature affect on physiological and behavioural activities of parasitoids and changes their rates of parasitism.

The field experiments described in this chapter further contribute to the information gained from previous chapters (4 and 6) that *A. subandinus* and *O. lepidus* respond to different densities of PTM. The results indicated that the two parasitoids had a high level searching capacity. This makes these parasitoids increasingly promising as biological control agents against the PTM.

Chapter Eight

Competition between *A. subandinus* and *O. lepidus*

"It is possible that interspecific competition between parasitoids could lead to reduced level of overall parasitisation and pest population regulation."

Ehler and Hall, 1982

8.1 Introduction

Competition between parasitoids may reduce the rate of total parasitism and regulation of a pest population (Watt, 1965; Ehler and Hall, 1982). However, differences in behaviour, interspecific host discrimination or reproductive ability (Chapter 4) could reduce competition between different species of parasitoids, and produce an overall greater suppressive effect (DeBach, 1966; Huffaker *et al.*, 1976; Keller, 1984; Hagvar, 1989; Van Albeek *et al.*, 1993). [IC1]Intra- and inter-specific competition among parasitoids may be separated into two categories, external, which occurs among adults in their search for hosts, and internal, which occurs among larvae for possession of a multiply parasitised host (Smith, 1929). The larvae of endoparasitoids are faced with two problems- firstly, host defence, and secondly, competition with other larvae (Schmid-Hempel and Schmid-Hempel, 1996). Intra- and inter-specific interactions between parasitoids have been studied by many researchers (e.g. Field, 1997; Losey and Denno, 1997). In some interactions parasitoids disrupt parasitism (i.e. total parasitism is lower than expected given the sum of parasitised hosts if each species acted in isolation; see Rosenhim *et al.*, 1995), while in other situations parasitoids facilitate parasitism (see Losey and Denno, 1997).

Competition between *A. subandinus* and *O. lepidus* may affect their local establishment and distribution (Franzmann, 1980; Flanders and Oatman, 1987; Horne, personal com., 1995). Competition is important in these solitary parasitoids as only one larva is able to complete its development in a single host. Investigation of competition between *A. subandinus* and *O. lepidus* may contribute to a better understanding of the combined efficiency of the two species in the parasitism of PTM (for more detail see Section 1.4.3.7). The role of interactions between *A. subandinus* and *O. lepidus* in regulating PTM populations is not clear. These experiments were conducted to investigate the interactions between the two species.

8.1.1 Aims

[IC2]The overall objective of this study was to examine how competition between *A. subandinus* and *O. lepidus* influences their coexistence on PTM.

The aims were:

1) to assess the relative success of *A. subandinus* and *O. lepidus* when offered a limited number of hosts;

2) to determine intra- and interspecific competition of the parasitoids and whether they compete at different developmental stage(s) and if so, which species would be successful;

3) to predict how releasing multiple species would impact on the PTM population in the field.

To address the above questions, two experiments were carried out, one in the laboratory and one in the field. In addition, observations were conducted during both experiments to identify the mechanism of competition. The development of the embryos of the two parasitoids and their morphological changes were observed to differentiate each species in multiparasitised hosts in a third experiment.

8.2 Competition between *A. subandinus* and *O. lepidus* when presented to hosts in different combinations

Competition between *A. subandinus* and *O. lepidus* was studied by exposing PTM larvae developing in potato foliage to the two parasitoids individually, simultaneously and sequentially. This experiment was carried out to investigate whether the egg-laying rate of assumed parasitoids changes when exposed to different combinations of PTM larvae.

8.2.1 Material and methods

To reduce the impact of differences between individual wasps in size, age, fecundity and previous experience, females of both species experienced the same conditions. Two- to three-day-old gravid, fed and experienced wasps (Section 5.2) were selected from the insect culture (Section 2.2). Container potato plants (Section 2.3) were each infested with 10 newly emerged PTM larvae. After 24hr, infested plants were exposed to the parasitoids in the following treatments for four hours:

A = *A. subandinus* alone,

O = *O. lepidus* alone,

A-O = *A. subandinus* followed by *O. lepidus* 4hr later,

O-A = *O. lepidus* followed by *A. subandinus* 4hr later,

A&O = *A. subandinus* and *O. lepidus.*

For each treatment, a pair of infested plants were placed in a cage (33 × 26 × 26cm). After this period, infested plants from each treatment were placed individually in rearing cages provided with potato tubers and white sand for larval feeding and pupation.

The data obtained on the emergence of the two species were analysed with a generalised linear model with binomial error to determine how the treatments affected the number of parasitised hosts in each treatment. Each situation was modelled individually to test for differences between the four treatment combinations. The model was: $\log(\frac{p}{1+p}) = 1 + treatment$,

where P is the proportion of parasitism.

Differences between the oviposition rates of the two species when they were introduced alone and when they were in a combination were analysed with t-tests using Genstat 5 (Lane and Payne, 1996).

8.2.2 Results

The mean (±sd) number of hosts established was 14.3±2.1 larvae on the two plants in all replicates. The mean of number of hosts parasitised per hour by *A. subandinus* was less than the number of hosts parasitised by *O. lepidus* in all treatments (Table 8.1).

Table 8.1: The mean (±sem) number of hosts parasitised per hour during 4hr by *A. subandinus* and *O. lepidus* when foraging alone or in combination (*A. subandinus* before *O. lepidus, A. subandinus* after *O. lepidus* and both together) in cages, n= 10.

Treatment	*A. subandinus*	*O. lepidus*	Combined parasitism
A	6.2±0.6	-	6.2±0.6
O	-	12.2±0.5	12.2±0.5
A-O	5.0±0.4	9.8±0.6	14.8±0.7
O-A	4.3±0.8	8.8±0.7	13.1±0.8
A&O	3.8±0.5	9.8±0.6	13.6±0.8

The mean number of hosts parasitised by *A. subandinus* and *O. lepidus* was higher when they were introduced alone than when they were in a combination with each other. The model showed significant differences between the oviposition rate of *A. subandinus* alone and in a combination ($t= 4.09$ and $P\leq 0.001$) and also the oviposition rate of *O. lepidus* alone and in a combination ($t= 4.02$ and $P\leq 0.001$).

When the two species were presented in any of the three combinations, there was no significant difference in the overall rate of parasitisation test. The overall rate of parasitisation

in the combinations was not significantly different from the oviposition rate of *O. lepidus* alone whereas, there was a highly significant difference between the oviposition rate of *A. subandinus* and the overall oviposition rate of the combinations (Table 8.2).

Table 8.2: The analysis of deviance test for total proportion between treatments.

Treatment	df	Residual Dev-	Pr (χ^2)
Total in (A-O, O-A, A&O)	47	73.70	0.07
A vs total in (A-O, O-A, A&O)	48	149.88	0.00
O vs total in (A-O, O-A, A&O)	48	75.07	0.24
A vs O in all treatments	49	151.68	0.00

8.2.3 Discussion

In general, the results show that *O. lepidus* was more successful in parasitising PTM larvae either alone or in competition with *A. subandinus*. As previous results on the fecundity of the two species showed (Chapter 4), *O. lepidus* females had a reproductive advantage over females of *A. subandinus*.

When the two species were introduced to the established hosts, the total number of parasitised hosts was higher than when *A. subandinus* was introduced alone. Various mechanisms allow the competing species to coexist and increase the rate of parasitism. For example, parasitising hosts at different developmental stages (see Briggs, 1993) or differences in some aspect of the environmental conditions (see Connell, 1978). In this regard, *A. subandinus* is probably most successful in parasitising newly emerged larvae because after 24hr PTM mine deeply inside the foliage, and becomes inaccessible to the female wasp which has a relatively short ovipositor. Furthermore, their host discrimination increases with time and after 24hr they reject parasitised hosts, and there are differences in the effects of temperature on development and longevity (Section 4.2.3.2).

Although there was no detectable increase in the rate of parasitisation when the two species attacked PTM in combination compared to *O. lepidus* alone, the presence of both species is desirable for biological control. The two wasps have different temperature tolerances so one could compensate for the other under certain environmental conditions. Moreover, if one species suffers unusually high mortality due to disease, hyperparasitism or another agent, then the other species would still have some suppressive action against PTM.

In conclusion, the results of this experiment indicated that releasing both *A. subandinus* and *O. lepidus* has a greater advantage than releasing only one species of the two parasitoids for managing PTM populations. As Godfray and Waage (1991) have shown, there are cases in which two parasitoid species are better at reducing the host abundance than a single species, if there is mutual interference in the attack rates of both parasitoid species.

8.3 Measuring host finding and oviposition capacity of *A. subandinus* and *O. lepidus* in a multiple release

The efficiency of the two parasitoids in host parasitism was investigated in a multiple release. Two experiments were conducted, one in the OWT and the other in the field. The objective of this study was to determine if females of both species parasitise PTM independent of the other in the population.

8.3.1 Material and methods

The OWT experiments

Sixteen container plants of equal size (Section 2.3) were infested with 2, 4, 8 and 16 newly emerged PTM larvae per plant with 4 plants for each density. After 24hr, the plants were set up randomly in the OWT arena. Three female wasps of each species were released in the OWT for 4hr. Experimental conditions in the OWT such as wind speed, temperature and

releasing technique were the same as in previous experiments (Section 6.2). At the end of each exposure time, plants were placed individually in rearing boxes (Fig. 2.1.B) until insects emerged. Observations were taken at 30min intervals during the period of each test to examine the interaction behaviours of intra- and inter-specific adult wasps during their searching and oviposition. This experiment was repeated four times under the same conditions.

Field experiments

Sixteen potted potato plants of equal size (30-40cm) were grown in the glasshouse (Section 2.3) and were infested with 10, 20, 40 and 80 newly emerged PTM larvae with 4 pots for each density. After 24hr, the pots were transferred to a field free of potatoes and parasitoids. The pots were set out as a completely randomised design around a circle 5m in diameter (Fig. 8.1). The circle was divided into four quadrants and in each quadrant four pots with four different densities were set up randomly. Four *O. lepidus* and four *A. subandinus* females of the same age (2-3-days-old) were collected from the insect culture. They were transferred into the releasing cage (Section 2.5) with an infested potato shoot for wasp experience. The releasing cage was placed in the centre of the circle of plants for 30min and then the first cover of muslin was removed to release the wasps. Females crawled from the holes on the second cover of the releasing cage to the top of the muslin. They then flew to the plants after a lag of few minutes and were allowed to search and oviposit for 6hr. At the end of this period, potato plants were returned to the insectary and the foliage of each pot individually collected for rearing of PTM larvae. The number of insects that emerged from the different host densities were recorded to estimate the rate of parasitism by the two species. This experiment was repeated six times on different days with temperatures between 22 to 27°C as recorded at the Waite Meteorological Station, The University of Adelaide, 200m from the experiment site.

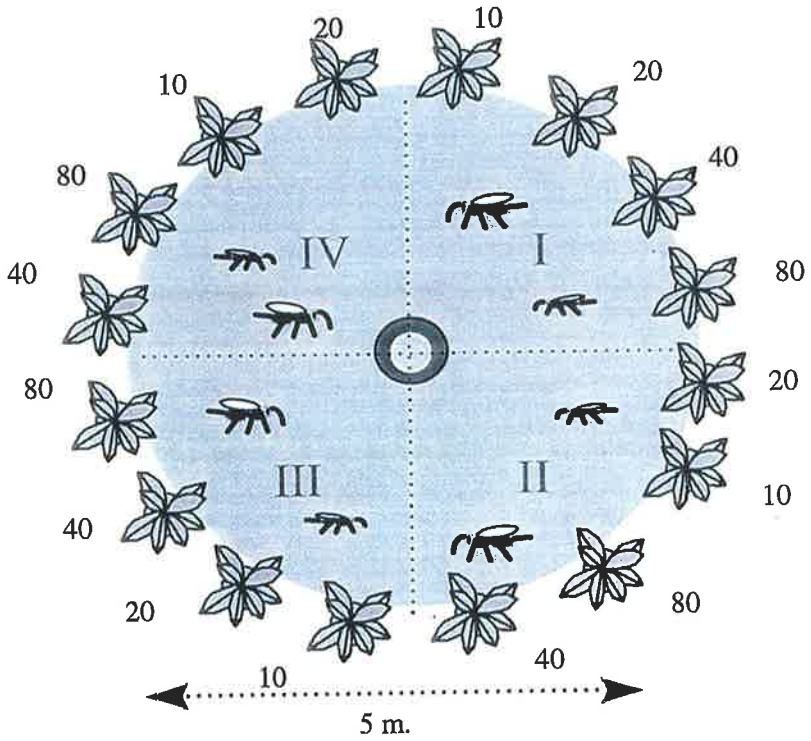


Figure 0.1: Photograph and design of the multiple release experiment in the field.

Data analysis

The data from each set of experiments in the OWT and in the field were analysed with linear regression using the Genstat 5 (Lane and Payne, 1996).

8.3.2 Results

The OWT experiments

Sixty four percent of hosts were established over four replications on 64 plants (i.e. 303 out of 480 larvae). From 222 parasitised larvae 82 *A. subandinus* (27%) and 140 *O. lepidus* (46%) adults emerged. The number of hosts parasitised by *O. lepidus* was comparatively higher ($P \leq$ 0.001) than *A. subandinus* at all densities (Fig. 8.2).

Field experiments

From 96 potted potatoes over six replications 1711 insects were collected. Thus, 47.5% of the original number (3600 larvae) were established. From these, 200 larvae were parasitised by *A. subandinus* (12%) and 584 larvae by *O. lepidus* (34%).

Generally, both species parasitised a higher number of hosts on plants that had a high density than on plants with a low density. Thus, the results from this experiment confirmed the data recorded in the density dependent experiments (Section 6.3.2) which indicated that the two parasitoids oviposited in more hosts in patches with high densities than low densities (Fig. 8.3).

The results from these experiments indicated significant difference ($P < 0.05$) between the number of hosts parasitised by *A. subandinus* and *O. lepidus* at all densities of PTM in the OWT and in the field.

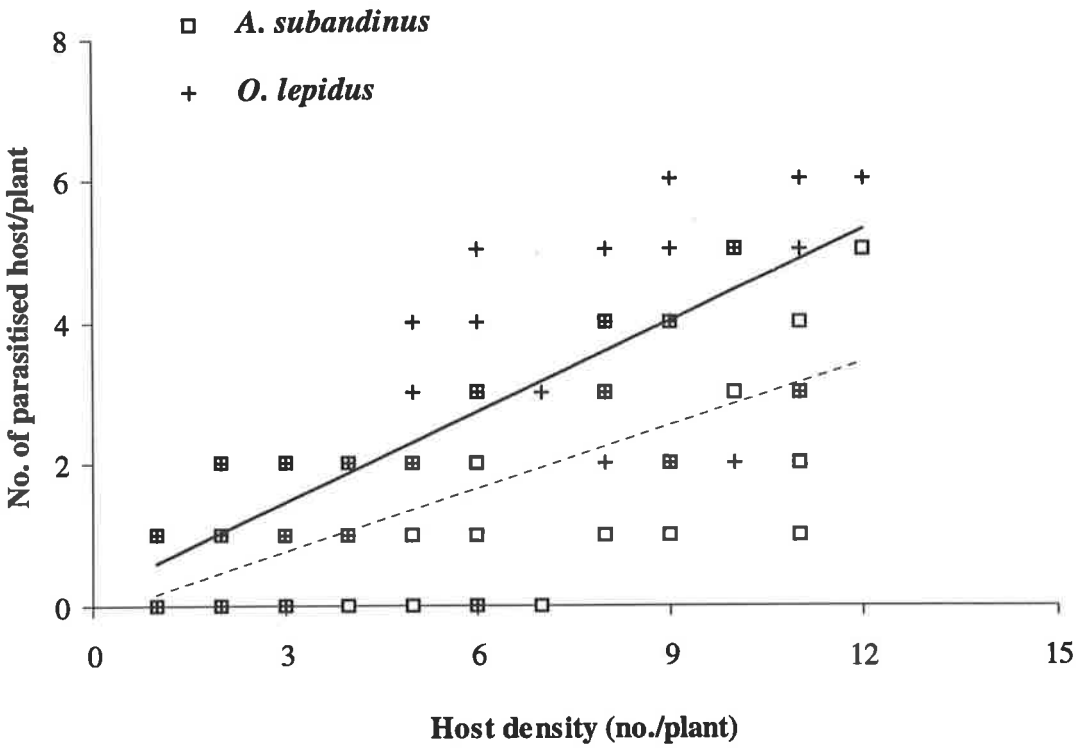


Figure 8.2: The number of hosts parasitised by three *A. subandinus* and three *O. lepidus* when released simultaneously in the OWT during 4hr exposure time, n= 12 for each density. Lines represent regression lines, solid for *O. lepidus* (R^2= 0.63) and broken line for *A. subandinus* (R^2= 0.52), ⊞ indicates data overlap for the two species.

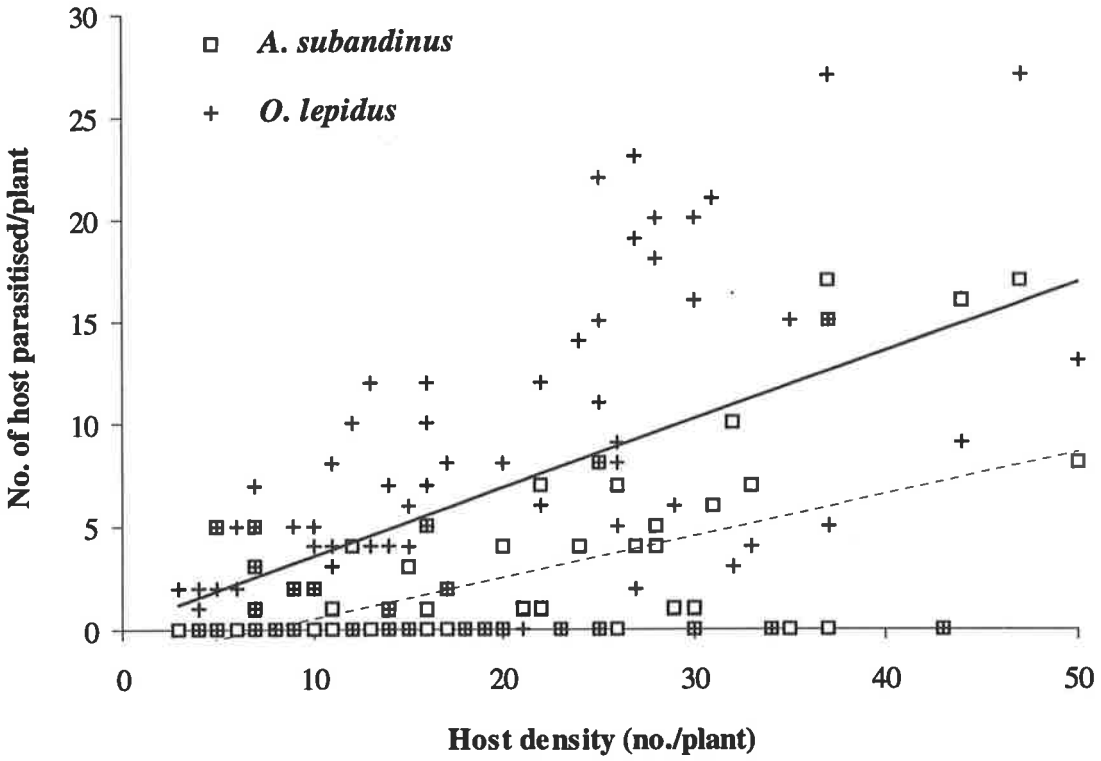


Figure 8.3: The number of hosts parasitised by four *A. subandinus* and four *O. lepidus* when released simultaneously in the field during 6hr exposure time, n= 24 for each density. Lines represent regression lines, solid for *O. lepidus* (R^2= 0.34) and broken line for *A. subandinus* (R^2= 0.30), ⊞ indicates data overlap for the two species.

8.3.3 Discussion

The results from both the OWT and field experiments suggested that the total number of hosts parasitised by the two parasitoids was higher when compared with the number of hosts parasitised by them individually. The number of hosts parasitised by *O. lepidus* was higher than by *A. subandinus* in all sets of experiments. This confirmed the previous results on the fecundity of the two species (Chapter 4).

There are many examples of coexistence of parasitoid species (see Rosenheim *et al.*, 1995). A closely related example is reported by Flanders and Oatman (1987), who found that *Apanteles scutelaris* and *Agathis gibbosa*, the two solitary endoparasitoids of PTM are able to coexist because of differences in their oviposition efficiencies relative to the location of PTM mines, and their host discrimination abilities. The results presented here confirm the previous results of Section 8.2.2, when *A. subandinus* and *O. lepidus* were introduced to hosts simultaneously in a cage.

8.4 Development of embryos and mechanisms of intra- and interspecific competition between *A. subandinus* and *O. lepidus*

Two individual experiments were conducted in this section. The first experiment was carried out to determine the developmental time of the embryos of the two parasitoids. The results of this experiment enabled identification of the eggs of the two species when they were released as a combination, "A-O, O-A and A&O" in Section 8.2.1. The second experiment was conducted to determine where the competition takes place; between adult parasitoids searching in the oviposition sites, among larvae of the parasitoids within the host, or in both stages. Finally, if they compete at the larval stage, this experiment would determine the mechanism of competition; whether one dominates the other through fighting or chemical suppression.

8.4.1 Material and methods

Experiment-1

In this experiment, 160 parasitised larvae (80 larvae of each species) were dissected at four intervals; 24hr, 36hr, 48hr and 60hr after oviposition by the two parasitoids individually. For dissection, each larva was placed in a drop of water in a clear plastic Petri dish on a black plate background of a stereomicroscope. A magnification of 40X was used and larvae were dissected using two forceps (Inox, No 4). The developmental stage (egg or larva) and morphological changes were recorded to determine the time after oviposition that was best for distinguishing between the two species.

The site of oviposition was also recorded for each species by gently opening the cuticle of the parasitised larva. Several superparasitised larvae were dissected to determine the locations of eggs laid in the host haemocoel after 36hr, 48hr and 72hr.

Experiment-2

The interaction of both adults and larvae of the two parasitoids were studied. The behaviours of adult wasps were observed during their host searching in a Petri dish (14cm diameter × 4cm; Fig. 3.1). In each test, an infested potato leaf with four larvae was provided in the dish and one female parasitoid of each species was introduced through a hole in the top. Female behaviours such as attack or retreat were noted. In addition, the behaviour of adults was observed when they had intra- or inter-specific encounters during the period of each experiment in the OWT.

The behaviour of the larvae of the two parasitoids was investigated in super- or multiparasitised larvae. These larvae were dissected at different times from the initial oviposition depending on information obtained from experiment-1 in this section. The

behaviour of the larvae was observed in 10% PBS (Section 4.3.1) under the stereomicroscope in the few minutes in which they were still alive. Each supernumerary larva was examined to find wounds or secretion of its hemolymph. A record was made for each observation on the two stages of parasitoids.

8.4.2 Results

Experiment-1

Larval dissections showed that both species were at the egg stage up to 24hr. However, changes in the morphology of *A. subandinus* were much clearer than those of *O. lepidus* and embryonic development showed enlargement in eggs of *A. subandinus* after 24hr (Fig. 8.4). Identification of the eggs of the two species was easier at this time. Development of the embryo was faster for *A. subandinus* and at 36hr after oviposition 85% of its eggs had hatched whereas *O. lepidus* was still at the egg stage (Table 8.3).

Table 8.3: Developmental period of embryo in *A. subandinus* and *O. lepidus* in the insectary (24°C).

no. of Larvae parasitised	Time	*A. subandinus*		*O. lepidus*	
		no. of eggs	no. of larvae	no. of eggs	no. of larvae
40	24hr	20	0	20	0
40	36hr	3	17	20	0
40	48hr	0	20	18	2
40	60hr	0	20	0	20

The eggs of both species are transparent, with an elongated shape and the surface of the chorion is smooth with one bluntly rounded end and a prominent pedicel that is directed towards the oviduct when the eggs are in the reservoir. Eggs that have been in a parasitised

host for 24hr change in shape, making identification of the two species easier (Fig. 8.4 and Fig. 8.5).

Females of both species deposit eggs in the haemocoel of the host attached by pedicels to the internal organs of the PTM larvae. Females of *O. lepidus* attach the egg to the gut (ventriculus), on the surface of the Malpighian tubules or to the trachea, whereas *A. subandinus* females attach their eggs mostly to the surface of gut or release them freely into the haemocoel (Fig. 8.4 and Fig. 8.5).

Experiment-2

Adult contests

Observation of all experiments on female wasps indicated that there was no aggressive behaviour or physical contests among females of the two species when they encountered each other. However, adults disturbed each other (intra- and inter-specific) when they came into contact with each other and immediately took flight.

Larval competition

Dissection of superparasitised and multiparasitised hosts revealed that there was no embryonic suppression in either parasitoid species. All eggs in superparasitised and multiparasitised PTM larvae hatched and their first instars were active at least 8 hours after hatching.

[IC3]Larval stages of *A. subandinus* and *O. lepidus* occur in the hemolymph of PTM larvae and both do not emerge from or kill hosts until the parasitoids pupate. The first instars of both species are mandibulate and sickle-shaped (Fig. 8.6). Their head is large in relation to the rest of the body and, particularly in *O. lepidus*, the head is dorsoventrally flattened.

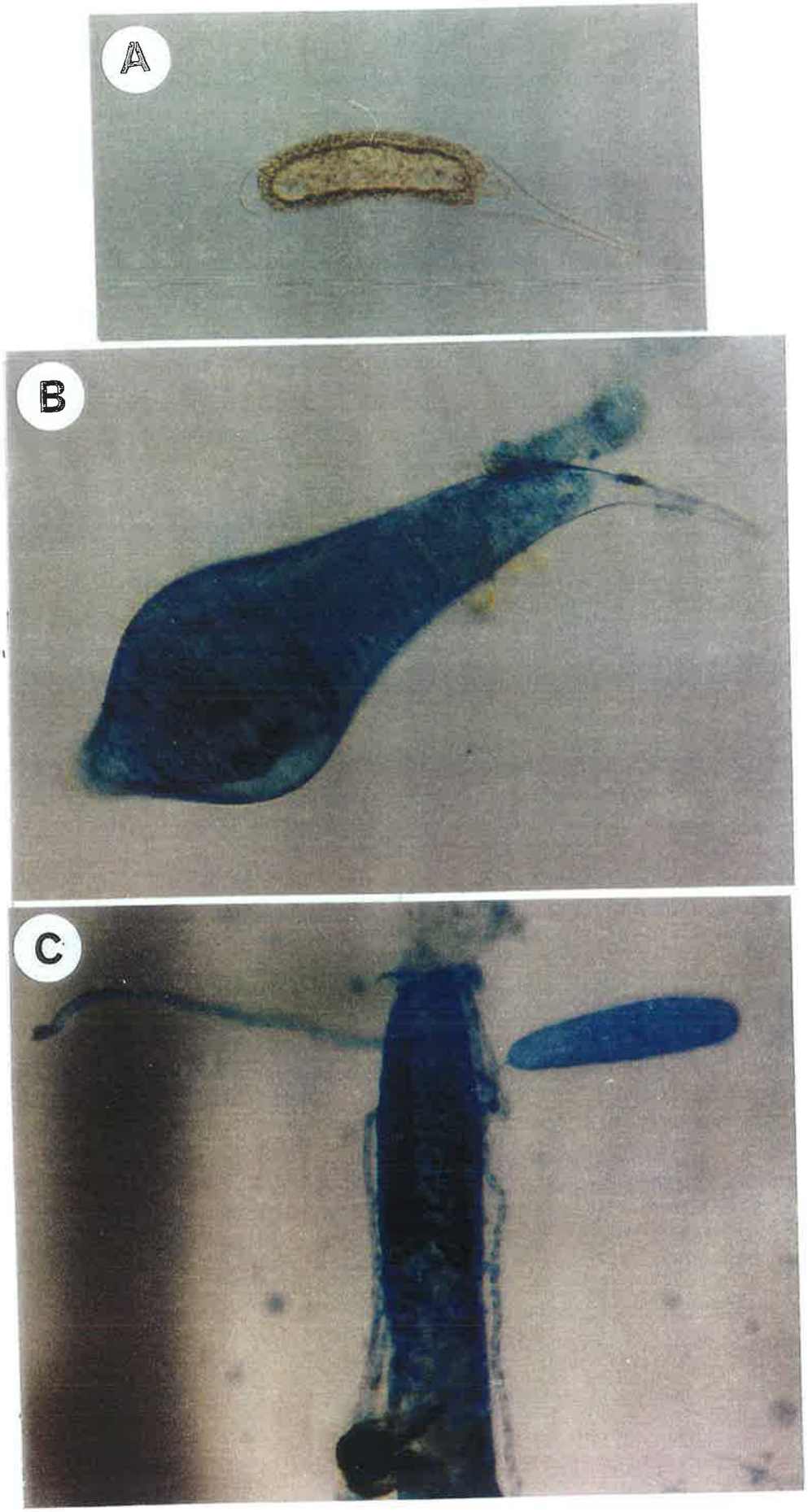


Figure 8.4: Eggs of *A. subandinus*, newly laid egg (**A**) and egg after 24hr (**B**), egg attached to gut of PTM larva (**C**) stained with methylene blue.

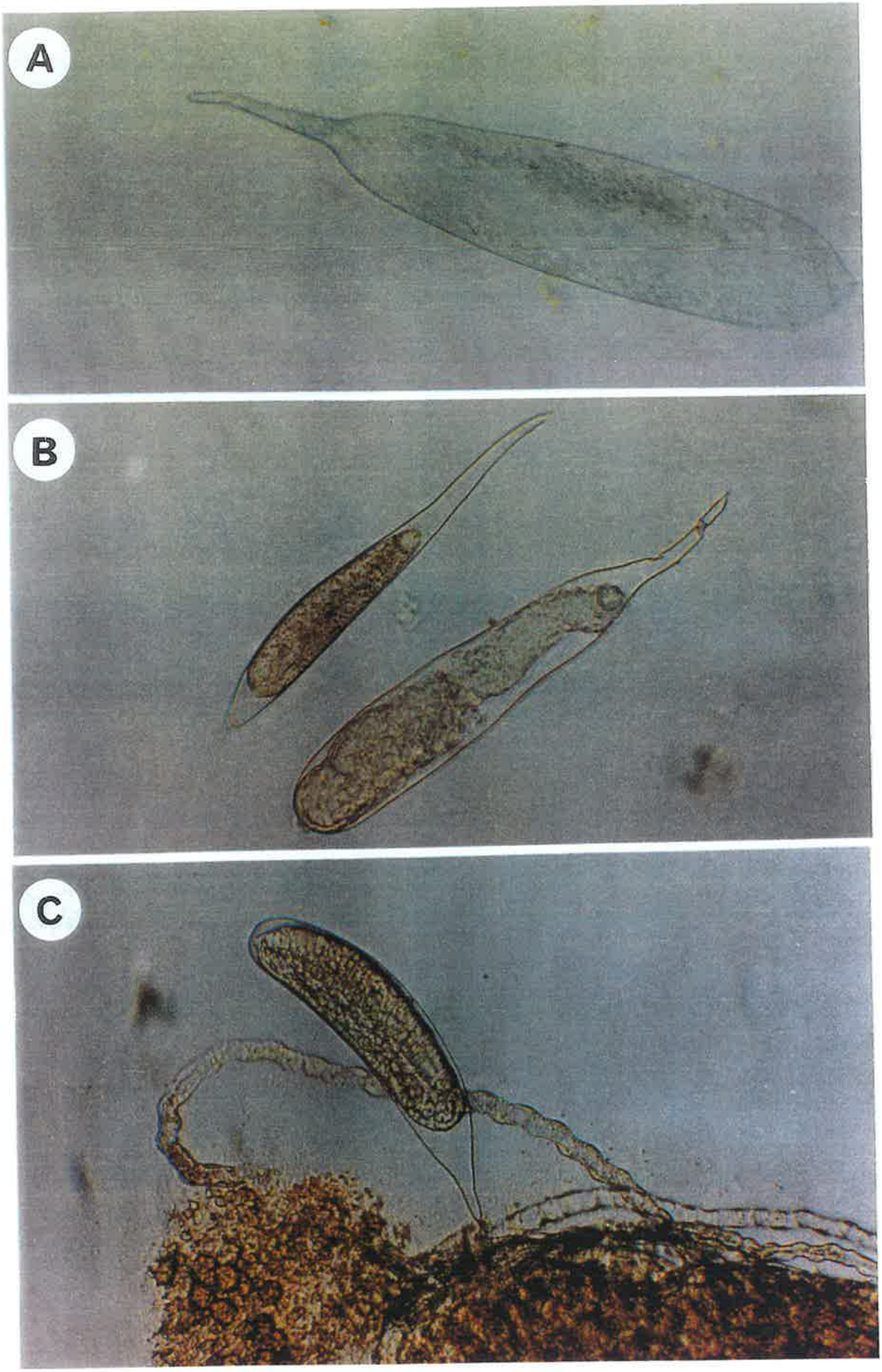


Figure 8.5: Eggs of *O. lepidus*, newly laid egg (**A**), eggs after 1hr (left) and 24hr (right) (**B**), egg attached to Malpighian tubules (**C**).

In superparasitised hosts, all parasitoid larvae were the same shape and size 8-12hr after eggs hatched but after one day one grew faster and the remainder of the larvae gradually degenerated (Fig. 8.6).

Results of many dissections of superparasitised larvae in self-, con- and inter-specific parasitised host at different times indicated that the larvae of parasitoids wounded each other. As a result physical attacks, wound marks and aggregation of hemolymph cells was observed around parasitoid larvae. Signs of physical attacks were observed on different parts of the dead larvae of parasitoids (Fig. 8.7). Biting of larvae was sometimes observed after superparasitised PTM larvae were dissected in PBS. This was observed for *A. subandinus* (40-50hr) after exposure to the wasp and for *O. lepidus* (50-60hr) and this fighting position remained for more than 15min in PBS solution. In several dissections, larvae of both species were observed with their mandibles buried in the body of a intra- or inter-specific superparasitised larva. One larva of a parasitoid (stronger one) in both species was biting on the sternum of a second which was biting a third. The dissection of multiparasitised hosts revealed that larvae of *O. lepidus* possess larger mandibles than those of *A. subandinus*. In most cases, regardless of which wasp parasitised the host first, *O. lepidus* killed the *A. subandinus* larva. The results may explain in part the outcome of experiment 1.

8.4.3 Discussion

Embryonic development

This study confirmed the results obtained in section 3.3 which indicated that *A. subandinus* grows faster than *O. lepidus*. The development of the first species was completed at least 12hr sooner than the second. These results helped to determine the best time for dissection of parasitised hosts for investigation when multiparasitism occurred at the same time.

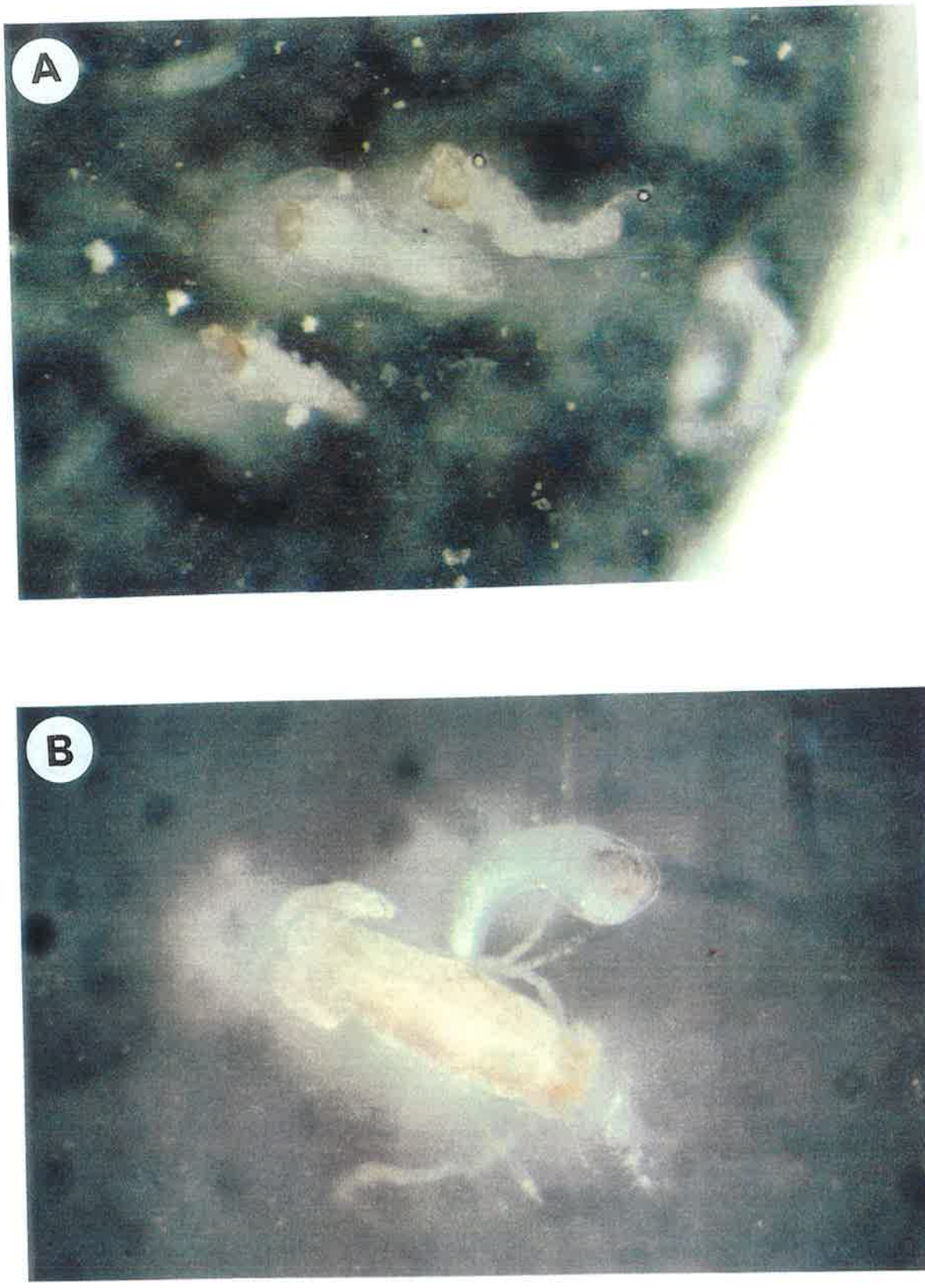


Figure 8.6: First instar larvae of *O. lepidus* in a superparasitised host of which only one was alive (**A**). Developing embryo of *A. subandinus* attached to the gut of its host (**B**).

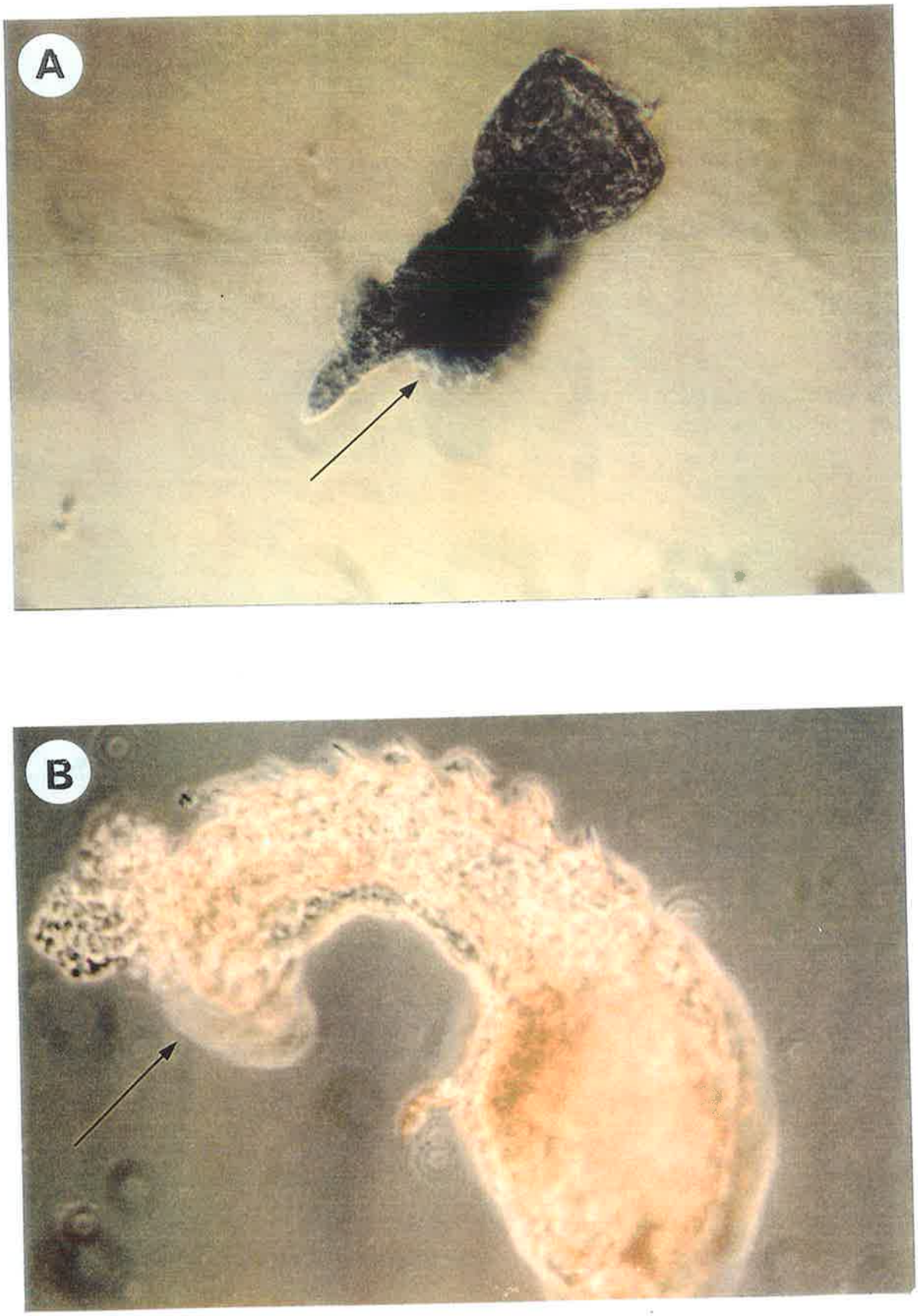


Figure 8.7: Wounded larvae in superparasitised hosts, a newly hatched larva of *A. subandinus* (**A**), and a newly hatched larva of *O. lepidus* (**B**). Arrows indicate wound sites.

Mechanism of competition

The female wasps did not show reactions toward one another when they were searching for hosts. These parasitoids exhibited no aggressive behaviour or physical contests that have been reported for some parasitoids such as *Trissolcus basalis* (Field, 1997).

In dissection of more than 500 PTM larvae superparasitised by *A. subandinus* and *O. lepidus*, it was found that all eggs hatched in the host but during the first instar only one larva developed quickly and the others gradually degenerated. All of the hatched larvae were alive within 10 hours of hatching in superparasitised hosts. After this time, most of them were dead and reduced in size except one live larva. Fighting between newly hatched larvae contributes to the regulation of numbers in these species. In cases of multiparasitism *O. lepidus* was the survivor if it occurred to 24hr after oviposition, apparently due to differences in the size of the mandibles of the larvae of the two parasitoids. If *O. lepidus* multiparasitised a host parasitised by *A. subandinus* 24hr before, the *O. lepidus* may be at a risk, because the larva of *A. subandinus* will be >50hr-old when larva *O. lepidus* appears.

Examples in previous research on Hymenopteran families show that the mandibles are used to bite and physically attack other larvae in the same host (Chow and Mackauer, 1984, 1986; Laing and Corrigan, 1987; Hubbard, *et al.*, 1987, Dijkerman and Koenders, 1988; Kfir and Van Hamburg, 1988). Hubbard *et al.* (1987) reported that when more than one larva of *Venturia canescens* (Grav) is present in a host, one of them bites the others and body fluids leak from the punctured skin of the injured larvae. This situation was also observed in some of the dead first instars of *O. lepidus* and *A. subandinus*.

Comparison of the size of eggs at different ages within hosts indicated that 24hr and 36hr-old eggs of *A. subandinus* and *O. lepidus* respectively increased in size. King *et al.* (1969) explained that layers on the pedicel region of the egg have a role in the adhesion of the egg to

the host organ, and act as an absorptive agent for nutrient substances. They found similar characteristics for *Cotesia glomerata.*

A maximum of six eggs was found in a multiparasitised host but, in no case was more than one second instar larva of a parasitoid seen in a dissected host larva that had been multiparasitised. Thus aggressive interactions must occur early in the development of the parasitoids. Mackauer (1990), suggested that the first-hatched larva of some Aphidiine wasps release a toxin to suppress younger competitors. Fisher (1963) also showed that the decrease in the oxygen content in the haemolymph of the host caused by the oxygen consumption of an older parasitoid *Venturia canescens* (Grav) was the crucial factor for the superparasitism of the younger parasitoids whose tolerance for low oxygen tension was much less than for the older larva.

In contrast Bakker *et al.* (1985) indicated that in solitary species, the second egg may have an advantage over the first one, because in long intervals between egg-laying by parasitoids, the larva hatched from the first egg could be at second instar before the second hatches. It is not mandibulate at this stage and can not fight with a first instar larva. In these parasitoids, super- or multi-parasitism occurred in short intervals and as described in section 9.3, the females mostly reject oviposition in hosts after 24hr.

As the competitive mechanisms are the same among members of one species, generally the oldest larva survives in competition with a younger one. But, observations in the previous experiment (this Section) showed that developmental times of embryos in a superparasitised host were very close to each other (2-8hr differences between hatching of first and last embryo).

In contrast to what was expected, *O. lepidus* was the superior competitor, even though the first instar larva of *A. subandinus* emerged on average 10hr before *O. lepidus*. The competitive

superiority of *O. lepidus* is perhaps due to the longer duration of its mandibulate first instar, (average 24hr) compared to that of *A. subandinus*. Furthermore, the first instar larva of *O. lepidus* is bigger and possibly stronger than *A. subandinus*.

Mackauer (1990) proposed that only one offspring of *Ephedrus californicus* an aphid parasitoid, will live and the remainder die in the egg or larval stage. Dissections of super- and multi-parasitised hosts demonstrated that when a PTM larva had more than one egg or larva, one larva eliminates its competitors and becomes the sole survivor. A degenerative appearance was also observed with a normal larva during interspecific competition of the two parasitoid species.

8.5 General discussion

The results of laboratory and field experiments indicate that *O. lepidus* is competitively superior to *A. subandinus* in both reproductive potential and larval competitive ability. The results of laboratory experiments showed that super- and multi-parasitism between conspecific or interspecific parasitoids causes loss of reproductive potential in both species. Particularly, the proportion of hosts attacked by *A. subandinus* was significantly lower when this species occurred in combination with *O. lepidus* compared to when it occurred alone. This was for two reasons; firstly, *O. lepidus* has a higher reproductive potential than *A. subandinus*, and secondly, the first instar larva of *O. lepidus* is stronger than *A. subandinus.*

Following release, one would expect wasps to parasitise host for many days. The advantage of releasing *A. subandinus* first would be minimal after day 1. Moreover, sequential release could require more labour.

Furthermore, the results from previous experiments indicated that there were differences in temperature dependent egg laying between the two species (Section 4.4.2). These differences enable establishment and coexistence of the two parasitoid in the field. This information

could be useful for making a successful decision for biological control of PTM by using a multiple release strategy for the two species which leads to a lower density of PTM compared to a release of a single species.

In conclusion, by combining the results from the reproductive ability of the two parasitoids, response to host density, their host discrimination and competition among larvae, it is suggested that multiple releasing of the wasps would be successful in biological control of PTM.

Chapter nine

Foraging decisions and host discrimination by *A. subandinus* and *O. lepidus*

Female *O. lepidus* probing and ovipositing a host in a mine

"Many parasitoids are able to distinguish between parasitised and unparasitised hosts, through the application of external or internal markers at oviposition"

G. Salt, 1937

9.1 Introduction

The ability to discriminate between unparasitised and parasitised hosts and to refrain from ovipositing in the latter has been shown for many parasitoids in most families of Hymenoptera (Van Alphen *et al.*, 1987; Van Lenteren, 1981). The ability of insect parasitoids to avoid a host previously parasitised by herself (self-host discrimination) or another member of the species (conspecific-host discrimination) or by a member of another species (interspecific-host discrimination) has been the subject of many investigations (Van Lenteren, 1981; Van Alphen and Nell, 1982; Turlings *et al.*, 1985; Van Alphen and Vet, 1986; Van Dijken and Waage, 1987; Vinson, 1991; Visser, 1993; Pijls *et al.*, 1995). When many females visit the same patch either simultaneously or sequentially, the risk of superparasitism can arise (Van Alphen and Visser, 1990; Van Alphen *et al.*, 1992; Visser, 1993).

The avoidance of oviposition in previously parasitised hosts is considered a desirable character for parasitoids in biological control (Van Lenteren, 1981; Waage and Hassell, 1982). Avoidance of self-superparasitism is common among parasitoids, and has been reported in over 150 species (Salt, 1961; Rogers, 1975; Van Lenteren *et al.*, 1978; Van Lenteren, 1981; Godfray, 1994). There are many examples of host discrimination by solitary endoparasitoids (Bakker *et al.*, 1972; Rogers, 1975; Hubbard *et al.*, 1987; Yamaguchi, 1987; Gates, 1993), all of which parasitise visible hosts (eggs or larvae). However, there have been few reports on the parasitism behaviour of parasitoids with hosts that are leaf miners (e.g. Greany and Oatman, 1972).

Whether to accept or reject a host that has already been parasitised is a foraging decision (Visser and Driessen, 1991). This decision may depend on chemical cues left by a previous female (Greany and Oatman, 1972). Bakker *et al.* (1985) found that host discrimination by parasitoids is not absolute. The female wasp is under physiological pressure to oviposit and

may not discriminate when an ideal host can not be located. However, when a choice is available the female often prefers to oviposit on the more suitable host. [IC1]When a parasitoid is contained in laboratory conditions with a few hosts, over time it will encounter fewer and fewer unparasitised hosts, and then it will often superparasitise them (Baker, 1976). Parasitoid females that are egg limited or do not gain any reproductive success by superparasitising should avoid superparasitism, because the cost may be high (Mackauer, 1990; Bakker *et al.*, 1985). A number of theoretical (e.g. Charnov and Skinner, 1985) and experimental (e.g. Visser *et al.*, 1990) studies have indicated that in certain circumstances, a parasitoid may increase its fitness by remaining in a patch and ovipositing into an already parasitised host.

Parasitoids discriminate by detecting external marking pheromones (Vinson and Guillot, 1972; Bosque and Rabinovich, 1979; Klomp *et al.*, 1980; Strand, 1986; Field, 1997), or an internal mark or change in the condition of the host (Jackson, 1966; Fisher and Ganesalingam, 1970). By using marks, a parasitoid can avoid repeated searches by discriminating already searched from unsearched sites. A female wasp can determine how well she has searched the patch when she encounters marks. Marks reduce intra- and inter-specific superparasitism by parasitoids (Vinson, 1984; Van Alphen and Visser, 1990). Internal discrimination by parasitoids depends on physiological changes within the previously parasitised host (Klomb *et al.*, 1980; Cloutier *et al.*, 1984; Chow and Mackauer, 1986; Strand, 1986).

Host discrimination is influenced by stimuli perceived both by the antennae and by the ovipositor. Several parasitoids leave chemical marks on surfaces they have searched which deter further searching. These marks originate as a pheromone secretion from the Dufour's gland (Vinson and Guillot, 1972; Greany and Oatman, 1972; Van Alphen and Nell, 1982; Sheehan *et al.*, 1993).

Greany and Oatman (1972), reported that *O. lepidus* marks areas they search and females spend less time in areas previously searched by conspecifics. These researchers extracted a pheromone from *O. lepidus* that was localised within the abdomen of the females. These researchers did not clarify whether *O. lepidus* females use chemical markers as a self- or conspecific-recognition cue.

9.1.1 Aims

The aim of this series of experiments was to determine the extent to which female *O. lepidus* and *A. subandinus* discriminate between healthy and parasitised PTM larvae. This study was concerned with searching behaviour, host discrimination and the conditions under which superparasitism occurred. The questions these experiments addressed were:

1) Is the behaviour of *O. lepidus* and *A. subandinus* females influenced by previously self, conspecific and interspecific parasitised hosts?

2) Does the time elapsed between exposures influence host acceptance and superparasitism by *O. lepidus* and *A. subandinus*?

3) Is the duration of searching on a plant infested with hosts affected by previous parasitism?

In this study six experiments were carried out in a wind tunnel to evaluate the host-searching and oviposition behaviour of *O. lepidus* and *A. subandinus*.

9.2 General materials and methods

To standardise conditions, in all tests wasps were placed into a cage (15 × 15 × 20cm) containing a container plant (Section 2.3) infested with 20 first instar PTM for 40min before releasing the wasp in the wind tunnel. This was carried out to allow wasp learning and also to maintain successful parasitisation of the host. Pilot tests were carried out to determine the

optimal time to leave the wasps in the cage to achieve the highest rate of parasitism and keep the number of superparasitised and unparasitised hosts to a minimum. The females of both species produced the highest number of offspring with this procedure (Appendixes 4.1 and 4.2).

Factors such as previous foraging experience with a host, or host food-plant, the presence of host-related materials, number and age of hosts and all other experimental conditions were constant. Therefore, in these experiments the influence of previous host parasitism was the only factor varied.

The duration of host finding, host recognition and ovipositional behaviour of female parasitoids in each experiment was recorded with an Olivetti computer programmed with event recording software (The Observer Version 2, Noldus Information Technology, Wageningen, The Netherlands). The wind speed in the wind tunnel was adjusted to 32.4cm/s, temperature in the wind tunnel was 24°C and light intensity was 4800 lux at the insect releasing site in all tests.

Larvae were collected after each experiment and dissected to determine the incidence of parasitism. For dissection, the procedure described in Section 8.4.1 was followed using a stereomicroscope with a magnification of 40X and two forceps (Inox, No 4).

9.3 Discrimination of plants with unparasitised and parasitised hosts from a distance

The first step in the investigation of host discrimination by female *O. lepidus* and *A. subandinus* was to determine whether they could discriminate from a distance between potatoes infested with parasitised and unparasitised hosts. Wasps were released in a wind tunnel to determine if they landed differentially on plants with parasitised hosts or unparasitised hosts (Keller, 1990).

9.3.1 Materials and methods

Potato shoots with three mature leaves were used for this experiment (Fig. 9.1.B). All shoots were kept fresh by immersion in water in a circular clear plastic container (150ml). Each shoot was infested with 15 newly emerged PTM larvae, 5 per leaf. Three prepared shoots were used in each experiment. After 24hr, shoots 1 and 2 were placed in a cage 15 × 15 × 20cm and exposed to four gravid females of the same age for 40min. This exposure minimised superparasitism, however, some superparasitised and unparasitised larvae were present in the leaves. Shoot 1 was kept as a control to determine the percentage of parasitism. At the end of the 40min exposure time, the larvae of the PTM on the control plants were collected and the number of parasitised and unparasitised hosts was recorded by dissection. Shoot 2 was placed into the wind tunnel with Shoot 3 bearing unparasitised hosts. Thus, in each test two infested potato shoots, one with previously parasitised larvae and the other with unparasitised larvae were used. The distance between the two shoots was 5cm, and from the wasp releasing vial to the shoots was 30cm. The shoots and the vial were both at 20cm from the floor of the wind tunnel (Fig. 9.1.D).

The three behaviours recorded in this experiment were; latency of flight, i.e. the time from release to initiation of flight; the duration of flight, i.e. time from take off to landing on a shoot at 30cm distance, and choice of landing on a shoot with parasitised or unparasitised larvae. Whenever females did not take off after 5min, they were removed from the wind tunnel and discarded. The test was terminated when females landed on plants.

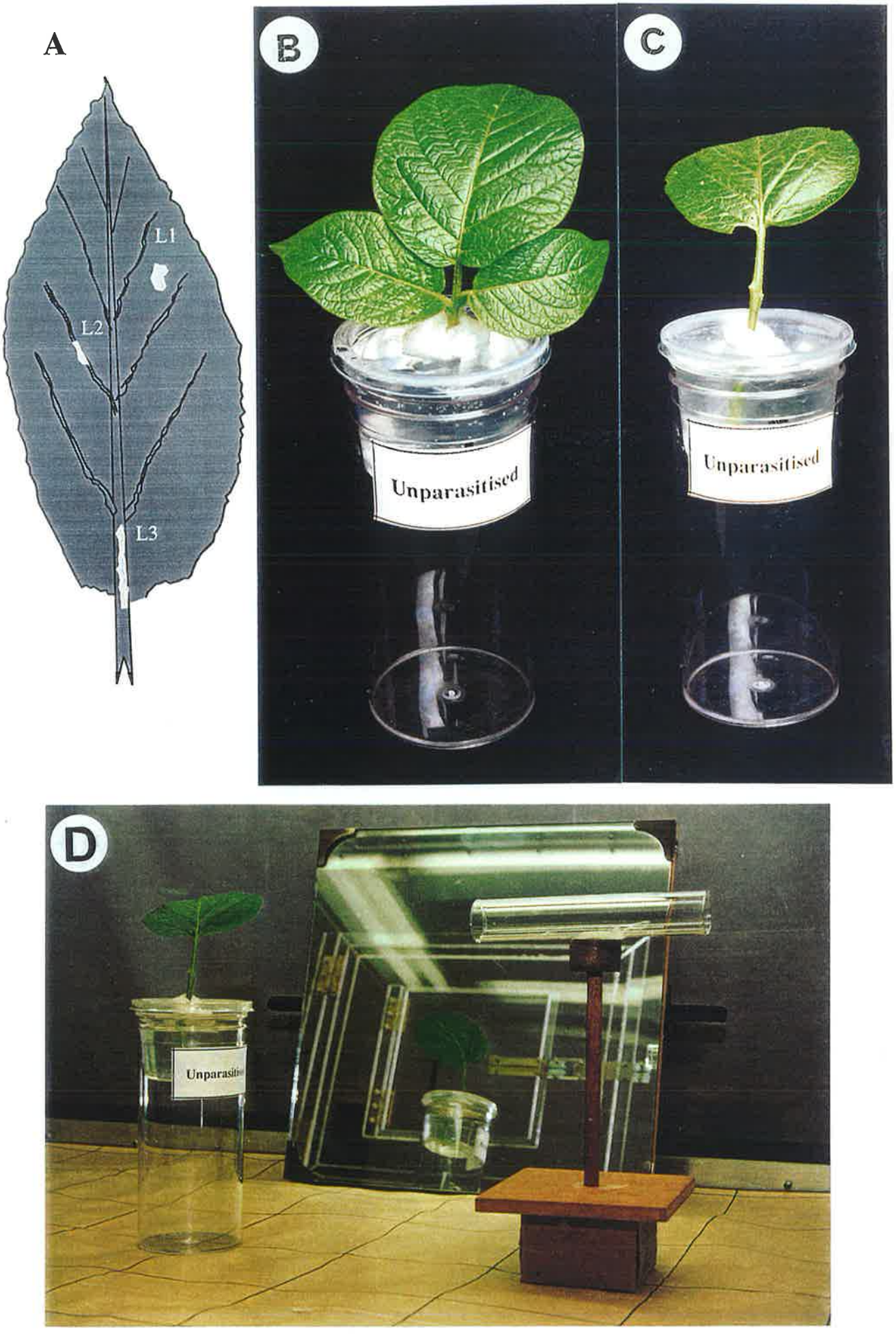
After the choices of all four wasps were recorded, the following procedure was done: 1) the collected larvae from shoot 1 (i.e. control) were dissected, 2) larvae of shoot 2 were reared until adults emerged and 3) larvae of shoot 3 (i.e. initially unparasitised) were discarded.

Figure 9.1: Apparatus used for host discrimination experiments:

A) Diagram of a leaf mined by three established larvae (L1, L2 and L3) with characteristic blistered appearance.

B) and **C)** A compound (centre) and a single (right) potato leaf in plastic cups (150ml), filled with water. Cotton was used to fix leaves in the holes in the lids. These leaves were infested with newly emerged PTM larvae.

D) The wind tunnel arena for observing behaviours of wasps. The wasp releasing vial was placed 20cm above the floor. A mirror was placed against the back wall of the wind tunnel to allow observation of behaviours of the wasps on the opposite side to the observer.


A
L1
L2
L3
B
Unparasitised
C
Unparasitised
D
Unparasitised

The patch selection experiment was replicated 10 times using a total of 40 females of both *O. lepidus* and *A. subandinus.* In each replicate four experienced females were released individually. The Binomial test (Zar, 1984) was performed using a computer program written by Keller (unpublished) to analyse the data. Standard errors are given for all means.

9.3.2 Results

Similar to the previous experiments (e.g. Sections 6.3 and 7.3), some PTM larvae did not establish on potato plants. Ten PTM larvae of a total of 15 (68 %) established on each plant. Only 12% of the larvae dissected from control plants were unparasitised after 40min exposure to 4 female *O. lepidus,* and 33% superparasitism was observed (Fig. 9.2). The estimated percent parasitism (±sem) based on dissections [87.3% (±3.4) n= 102] was similar to that obtained by rearing [90.5% (±2.7) n= 84].

Twenty eight out of 40 female *O. lepidus* selected the plant with unparasitised hosts compared with those with hosts parasitised 2hr before and analysis indicated wasps landed differentially on the two types of plants (P= 0.001). From 40 released wasps, 19 female *A. subandinus* landed on plants with unparasitised hosts, 14 females landed on plants with parasitised hosts and there was no significant preference for this parasitoid (P= 0.243) (Fig 9.3).

Latency of flight by *O. lepidus* ranged from 1.7s to 235s with a mean of 41.9s (±7.1) and tended to be longer when females flew to the plant with unparasitised hosts (Fig. 9.4). However, there were no significant differences between the mean times of latency when females flew to plants with unparasitised or parasitised hosts (P= 0.31, t-test, Zar, 1984). The duration of flight of female *O. lepidus* varied from 1.2s to 11.7s with a mean of 3.8s (±0.4) for 40 females and for *A. subandinus* varied from 1.3s to 6.8s with a mean of 2.1s (±0.6). The means of flying times were not significantly different when females landed on either type of plant.

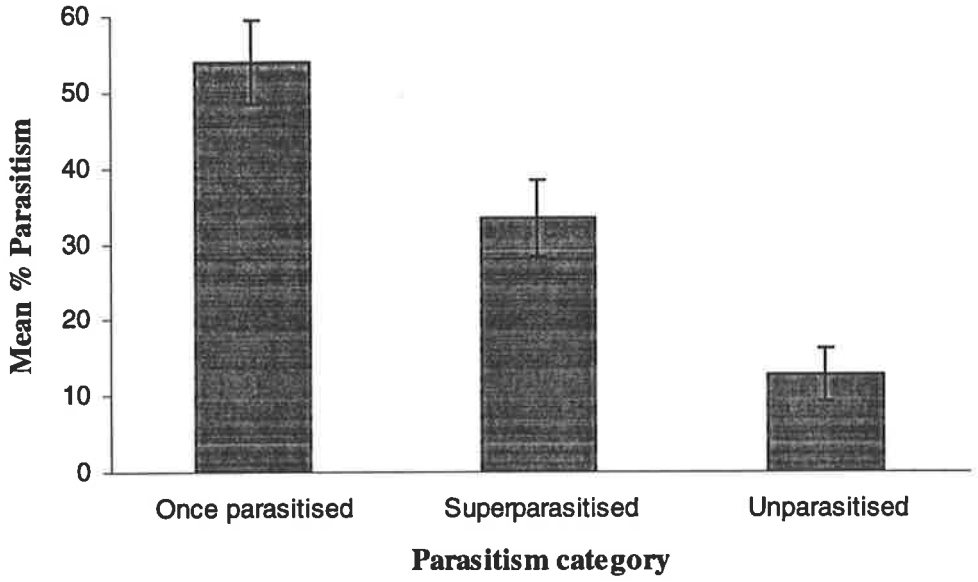


Figure 9.2: The mean percentage of parasitism of 10.1±2.9 PTM larvae by 4 female *O. lepidus* over 40 minutes. (n= 10).

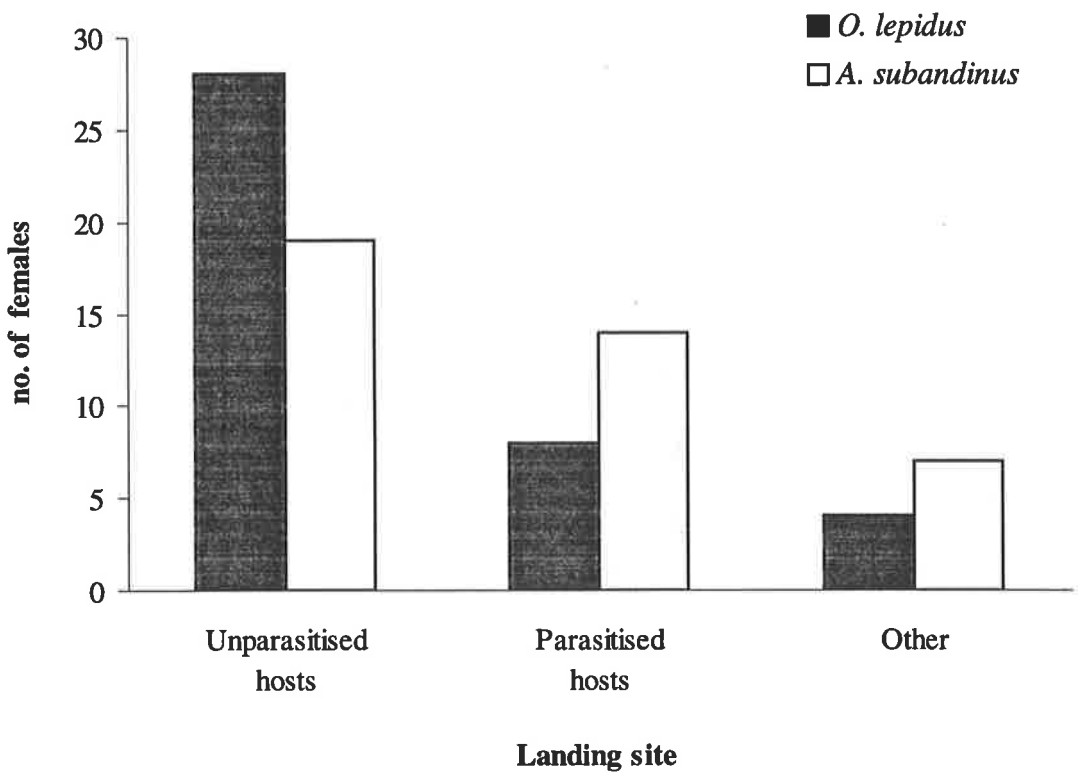


Figure 9.3: Choices made by *A. subandinus* and *O. lepidus* females between unparasitised and parasitised hosts and other sites in a flight tunnel (n= 40 for each species). *O. lepidus* preferred leaves with unparasitised hosts (Binomial test, $P< 0.05$) while *A. subandinus* showed no preference ($P> 0.05$).

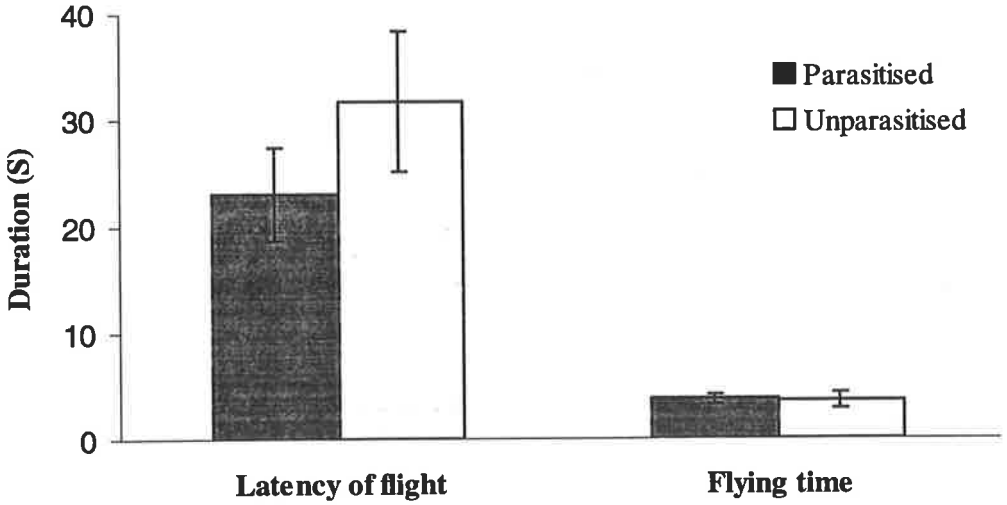


Figure 9.4: Comparison between duration of latency of flight and flying time of *O. lepidus* females that landed on plants with parasitised and unparasitised hosts (n= 14, *P*= 0.31).

9.3.3 Discussion

Flying *O. lepidus* females were able to discriminate between hosts parasitised 2hr before and unparasitised hosts, whereas *A. subandinus* females landed without preference on plants with unparasitised hosts and hosts parasitised 2hr before by self and conspecifics. Thus female *O. lepidus* could discriminate between plants with unparasitised hosts and hosts parasitised 2hr before. Female *A. subandinus* lack this ability.

It is not clear if the response of *O. lepidus* indicates a preference for unparasitised hosts or an avoidance of parasitised hosts. Moreover, the mechanism is unknown. It is possible that the wasps leave a volatile mark which is detected by females in flight. However, a volatile mark would be lost over time due to evaporation and hence its effects would be transient. It is more likely that the behaviour of parasitised larvae changes. Plant damage caused by host feeding is thought to be the primary source of kairomones that attract *O. lepidus* (Keller and Horne, 1993). If parasitised larvae feed less following parasitisation, then they should be less

attractive to their parasitoids. As *A. subandinus* did not respond differentially to parasitised and unparasitised larvae, it appears unlikely that parasitism alone could account for such a change in behaviour. Perhaps there is a greater reaction by host larvae to parasitism by *O. lepidus*.

Behavioural observations of host larvae following parasitisation by the two species and additional choice tests at various times following parasitisation should indicate the mechanism of this discrimination by flying *O. lepidus*.

9.4 Self and conspecific superparasitism

The aim of this experiment was to test the extent to which females of *O. lepidus* and *A. subandinus* superparasitise hosts that were parasitised less than 1hr before by themselves (self host discrimination) or by another female (conspecific host discrimination).

9.4.1 Materials and methods

Individual tests were carried out for the two species in the wind tunnel and behaviour was recorded using the Observer Version 2 (Noldus, 1991). For *A. subandinus*, 10 replications and, for *O. lepidus*, 20 replications were conducted under the same conditions as the previous experiment (Section 9.3.1) (i.e. the age of mines, the age of the wasps, feeding conditions and the wasp experience). Each replication was conducted with three treatments, PTM larvae once exposed to a wasp (control), PTM larvae exposed to the same female wasp twice (self) and PTM larvae exposed sequentially to two different females (conspecific).

A second experiment was conducted on conspecific host discrimination by *O. lepidus* with 15 replicates. The procedures were the same as those for the first set of experiments on conspecific host discrimination, except an extra observation was taken to clarify from which surface (upper or lower) of the leaves females oviposited during the first and second exposure

periods when they superparasitise a host. The behaviour of the female wasp was recorded on the diagram of the infested leaf for each larva attacked (Fig. 9.1.A).

The size of potato shoots was different from the previous experiment (Section 9.3.1). In each test, three mature potato leaves were fixed individually in circular clear plastic containers filled with tap water (Fig. 9.1.C). Each leaf was infested with four newly-emerged PTM larvae and held for one day until the larvae developed and established on the plant. Leaves with three established PTM larvae were selected for the tests.

In this experiment, three common oviposition behaviours, antennating, shallow probing and deep probing were recorded for each female for a maximum of 20min. The term antennating was used when females tapped damaged areas on the leaf with the terminal segments of their antennae during searching on the leaf. This behaviour often continued until they contacted a mine from the surface of the leaf and they then commenced probing. The term probing was used to describe jabbing the surface of the infested plant with the ovipositor after female wasps had detected the location of a host or host effects. Probing was divided into two categories, shallow and deep probing. Shallow probing involved jabbing the leaf quickly without inserting the whole length of the ovipositor into the plant tissue. Deep probing described when females inserted the whole length of their ovipositor into the mined area. The duration of ovipositor insertion and number of insertions were dependent on the length of the tunnel dug by the host. It was impossible to determine by observation whether a female actually oviposited in the host.

In each test, four gravid and fed females were used. They were transferred simultaneously to a cage (15 × 15 × 20cm) containing an infested potato plant with 20 unparasitised hosts, 1hr before starting the tests in the wind tunnel.

Firstly, one of the four females was chosen randomly and released in the wind tunnel to parasitise hosts on leaf 1 (control). The searching behaviour of each female and her encounters with each individual host were recorded. The first female and leaf were removed after the wasp appeared to oviposit in all of the larvae on the leaf.

In the second step, female 2 was introduced to the second infested leaf and after encountering all available hosts, she was removed and kept in a numbered cage for 30-45min until a second period of exposure to hosts previously parasitised by herself.

In the third stage of the test, female 3 was introduced to leaf 3. After recording oviposition behaviour on each larva, she was removed.

In the fourth stage, female 2 was introduced for a second period to self parasitised hosts on the second leaf. Finally, female 4 was introduced to leaf 3 with hosts parasitised by female 3 to investigate conspecific host discrimination. Female 4 was removed after encountering all the available hosts. The observation was stopped after recording the last oviposition behaviour.

The infested leaves were held in separate cages to allow the parasitoid eggs to partially develop. All of the PTM larvae were dissected 24hr after the completion of the tests to determine the proportion of those attacked that contained a single egg (parasitised) or more than one egg (superparasitised).

Total parasitism was estimated by combining the number of hosts parasitised with the number superparasitised for each individual female. The mean percentage of parasitism by wasps was estimated by the formula (number parasitised/total number of established hosts) × 100 and the mean percentage of superparasitism by wasps was also estimated (number of superparasitised hosts/total number of hosts) × 100.

Analysis

The relative frequencies of unparasitised, once parasitised and superparasitised PTM for the two parasitoids were compared in a contingency table analysis (Lane and Payne, 1996) to determine if the overall distribution of parasitism and superparasitism differed among the treatments. In further analysis, the observed parasitism was compared to the expected parasitism if wasps oviposit at random. If wasps do not avoid superparasitism, then the observed frequencies of number of eggs per host should not differ from a Poisson distribution. However, if the frequency of once parasitised host was greater than expected and the frequency of superparasitism correspondingly less, then host discrimination would be implicated. The Poisson distribution was used to calculate the expected parasitism in three ways. 1) The mean of the Poisson distribution was estimated from the observed frequency of unparasitised hosts. 2) The mean of the Poisson distribution was estimated from the observed frequency parasitised hosts (number of once parasitised plus 2X twice parasitised hosts divided by the number of hosts). 3) The number of hosts parasitised in the first exposure was estimated by multiplying the observed frequencies in the control by the ratio of total hosts in the treatment divided by the total hosts in the control. The total number of additional eggs laid in these hosts during the second exposure was then estimated by subtracting the number of eggs/host in the control from the number of eggs/host in the treatment. Finally, these additional eggs were distributed at random and added to the estimated parasitism at the beginning of the second exposure. The first two methods examined overall patterns of parasitism, while the third specifically addressed the distribution of parasitism by the second female.

Also, the numbers of ovipositions in the first and second exposures were compared. If all things are equal, then the numbers of eggs laid in hosts exposed to parasitoids twice should be double to that of the control if superparasitism is not affected by the presence of parasitised

hosts. Alternatively, a drop in oviposition rate in the second exposure would indicate restraint by the second wasp, and host discrimination would be implicated.

9.4.2 Results

The results of the dissection of the PTM larvae collected from the different tests indicated that both parasitoids superparasitised hosts in the three treatments (control, self and conspecific). The total percent parasitism (i.e. including once parasitised and superparasitised) by *A. subandinus* (n= 112) was 89.6%. The percentage of superparasitism by *A. subandinus* increased from the control (23%) to self (38%) and to conspecific treatments (44%) (Fig. 9.5). The total percent parasitism by *O. lepidus* (n= 149 in the first experiment and n= 39 in the second) was 90.0% and 91.5% respectively. The percentage of superparasitism by this species increased from the control to self and to conspecific. However, the rate of superparasitism by *O. lepidus* was lower in the control and self than *A. subandinus* (Fig. 9.6).

Contingency table analysis indicated that the distribution of parasitism differed among the treatments for *O. lepidus* but not *A. subandinus* (Table 9.1). Further analysis indicated that the difference for *O. lepidus* was due to a change in the distribution of parasitism in the conspecific treatment. This suggested that both species may avoid superparasitism as an increase in superparasitism and a decrease in the numbers of once parasitised hosts would be expected in the self and conspecific treatments if they did not discriminate against parasitised hosts.

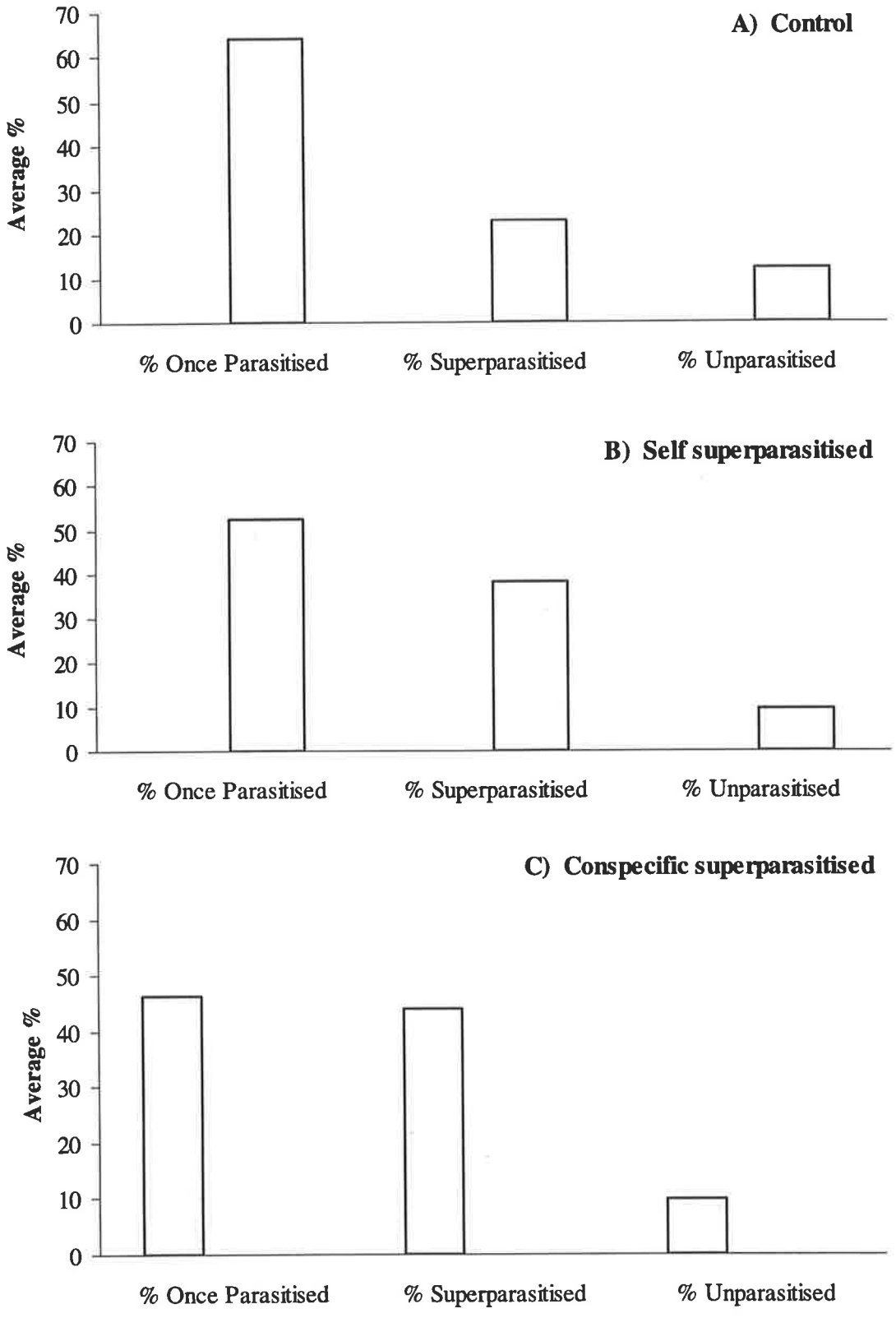


Figure 9.5: Parasitism by *A. subandinus,* when: **A)** an individual female was introduced once, **B)** the same female was reintroduced 30-45min after the first exposure, and **C)** when a conspecific female was introduced 30-45min after the first female.

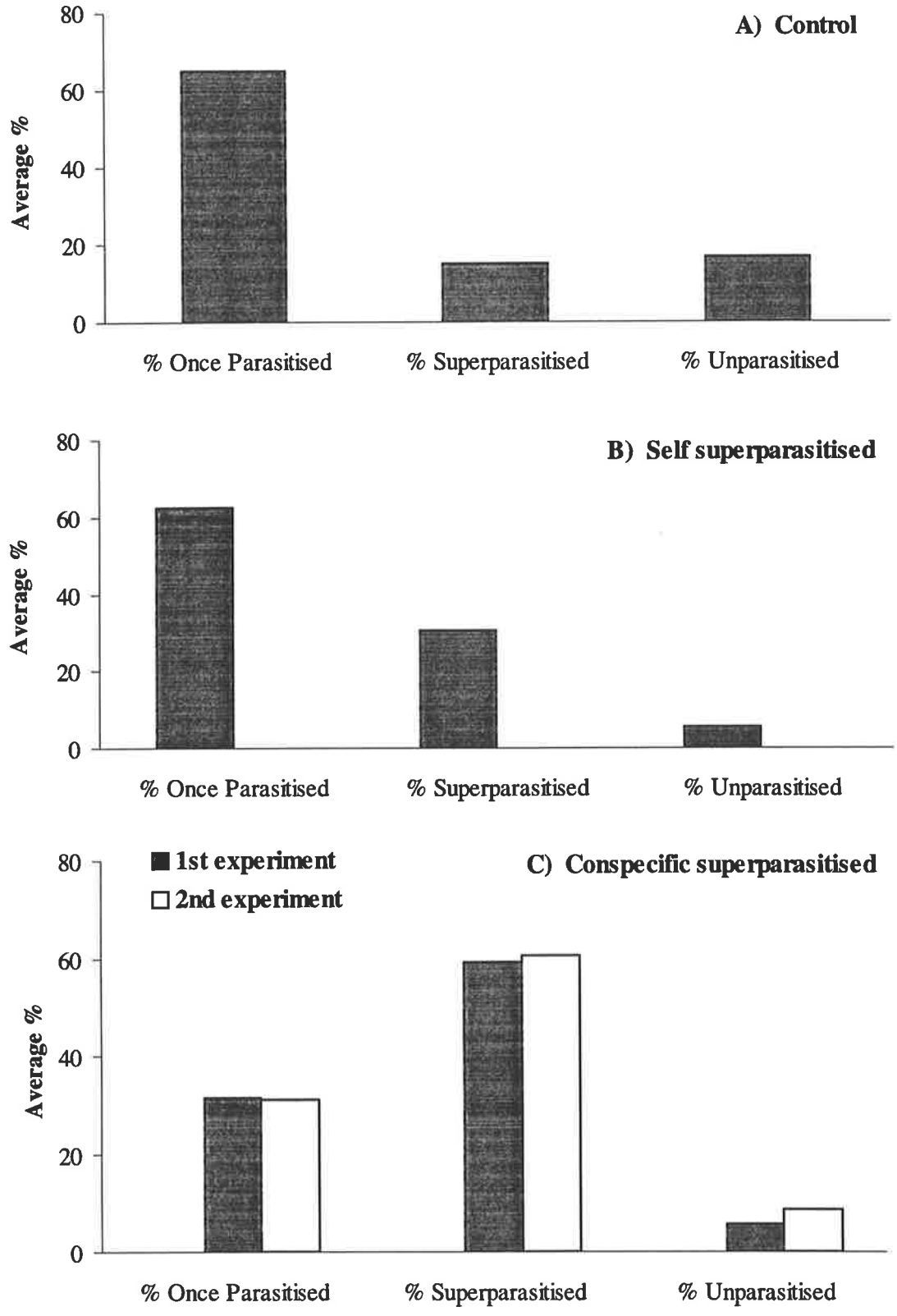


Figure 9.6: Parasitism by *O. lepidus* when: **A)** an individual female was introduced once, **B)** the same female was reintroduced 30-45min after the first exposure, and **C)** when a conspecific female was introduced 30-45min after the first female.

Table 9.1: Contingency table analysis of categories of parasitism when wasps were introduced to previously parasitised hosts (Chi-square test, using Genstat 5).

Treatments	Analysis value	Overall comparison of 3 treatments	Self vs control	Conspecific vs control	Self vs conspecific
O. lepidus	*P*	0.001	0.174	0.001†	0.001†
	χ^2	23.41	1.85	19.67	12.05
	df	2	1	1	1
A. subandinus	*P*	0.148	0.259	0.280	0.570
	χ^2	3.82	1.27	2.82	0.32
	df	2	1	1	1

† Statistically significant ($P \leq 0.05$) when probabilities are adjusted for multiple comparisons using the Sequential Bonferroni method (Rice, 1989).

The host discrimination behaviour of the parasitoids was further elucidated by comparing the frequency distribution of eggs in hosts to that expected if ovipositions were random. The first and second analyses of data obtained for *O. lepidus* and *A. subandinus* indicated that the distributions were different from what was expected if random oviposition occurred in all three treatments. In all cases, the frequency of once parasitised hosts was greater than expected and the frequency of superparasitised hosts was less than expected, suggesting an avoidance of superparasitism. However, there was little opportunity for wasps to superparasitise hosts in the control treatment, so these analyses are not conclusive.

In the third analysis, the results indicate that the distributions of eggs laid by the second female did not differ from what was expected if oviposition by the second female was random in either the self or the conspecific treatments (Table 9.2). Virtually the same results were obtained for *A. subandinus* (Table 9.3). Thus no evidence was found with this analysis that wasps were avoiding superparasitism during the second exposure. However, this analysis was probably not statistically powerful as few eggs were laid during the second exposure.

Table 9.2: Analysis of categories of parasitism by *O. lepidus* when females were exposed to hosts previously parasitised by then self or a conspecific wasp (SAS, 1995). In this analysis, the observed parasitism was compared to the expected parasitism if wasps oviposited at random. The Poisson distribution was used to calculate the expected parasitism using three methods (see Section 9.4.1).

Method 1: Expected frequencies of parasitism estimated from the observed frequency of unparasitised hosts (Analysis of once and superparasitised categories only).

Method 2: Expected frequencies estimated from the mean number of eggs laid per host.

Method 3: Expected values are derived from the estimated number of eggs laid by the second female if oviposition was random (i.e. second exposure of self or conspecific).

		Observed values	
Treatment	**Unparasitised**	**Once parasitised**	**Superparasitised**
Control	10	39	9
Self	3	35	17
Conspecific	3	17	32
		Method 1	
		Expected values	
Control	10.0	17.6	30.4
Self	3.0	8.7	43.3
Conspecific	3.0	8.6	40.4
Chi-squared probability of this result:		Control	<0.001
		Self	<0.001
		Conspecific	0.001
		Method 2	
		Expected values	
Control	21.7	21.3	15.0
Self	15.7	19.7	19.6
Conspecific	11.0	17.1	24.0
Chi-squared probability of this result:		Control	<0.001
		Self	<0.001
		Conspecific	0.014
		Method 3	
		Expected values	
Self	7.2	30.1	17.6
Conspecific	1.6	19.5	30.9
Chi-squared probability of this result:		Self	0.194
		Conspecific	0.448

Table 9.3: Contingency table of categories of parasitism by *A. subandinus* (see Table 9.2 for details).

Treatment	Unparasitised	Once parasitised	Superparasitised
Observed values			
Control	5	27	10
Self	4	22	16
Conspecific	4	19	18
Method 1 Expected values			
Control	5.0	10.6	26.4
Self	4.0	9.4	28.6
Conspecific	4.0	9.3	27.7
Chi-squared probability of this result:		Control	<0.001
		Self	<0.001
		Conspecific	<0.001
Method 2 Expected values			
Control	13.7	15.3	12.9
Self	11.6	14.9	15.5
Conspecific	10.7	14.4	15.9
Chi-squared probability of this result:		Control	<0.001
		Self	0.015
		Conspecific	0.050
Method 3 Expected values			
Self	4.2	23.6	14.2
Conspecific	3.1	17.9	19.9
Chi-squared probability of this result:		Self	0.842
		Conspecific	0.774

Analysis of the overall number of eggs laid per host provided the most compelling evidence of host discrimination (Table 9.4). Both species laid fewer than the expected doubling of those laid in the control treatment. In the case of *A. subandinus*, so few eggs were laid during the second exposure that the additional numbers could not be detected statistically. The drop in oviposition at the second exposure indicated restraint by the females in the second exposure and host discrimination is implicated. Significantly, *O. lepidus* laid fewer eggs when a female encountered hosts she had parasitised previously compared to those parasitised by a conspecific. This indicates a greater avoidance of self superparasitism than conspecific

superparasitism, but conspecific superparasitism is also avoided. Overall the analyses indicated that both species avoid superparasitism to some extent.

Table 9.4: Analysis of the numbers of eggs laid per host. For each species means followed by the same letter did not differ significantly (SNK test).

				95% Confidence interval	
Species	**Treatment**	**Sample size**	**Mean**	**Lower**	**Upper**
O. lepidus	Control	20	0.98 **a**	0.81	1.15
	Self	20	1.26 b	1.06	1.46
	Conspecific	20	1.53 c	1.33	1.74
A. subandinus	Control	14	1.12 a	0.94	1.30
	Self	14	1.30 a	1.12	1.48
	Conspecific	14	1.35 a	1.18	1.51

The information collected from host location on the leaf mine diagram (Fig. 9.1.A) before exposure to wasps and superparasitised hosts in each mine indicated that 88% of superparasitised hosts were located inside long mines in the veins. This indicated that host position had important effect on the rate of superparasitism by the two species.

9.4.3 Discussion

There was superparasitism by both parasitoids when they encountered hosts parasitised by themselves a few minutes before in the control treatment. Most of the superparasitism observed in this experiment occurred during the first exposure to wasps suggesting that discrimination is weakest for newly parasitised hosts. This result conflicts with the prediction of Visser *et al.* (1992a&b) that solitary parasitoids should never superparasitise when they search a patch alone.

Females of *O. lepidus* partially avoided superparasitism within 1hr of initial oviposition and self discrimination was stronger than conspecific. *A. subandinus* laid few eggs in either the self or conspecific treatments, indicating host discrimination in both cases. Thus *O. lepidus* has a lesser ability of host discrimination than *A. subandinus* at less than one hour. This discrimination should lead to more efficient foraging by both species.

9.5 Time-dependence of host discrimination

An experiment was carried out to examine the discrimination behaviour of *A. subandinus* and *O. lepidus* at different time intervals up to 24hr after the first parasitisation of PTM larvae. The aim was to investigate if the responses of the female parasitoids to previously parasitised hosts by self or conspecifics vary with the interval of time after the initial exposure.

9.5.1 Materials and methods

To examine self-host discrimination of the parasitoids at different exposure times, three leaves, each infested with three hosts similarly to experiment 2 (Section 9.4.1) were separately exposed to the same female at different time intervals. Three- to 5-day-old experienced females of *O. lepidus* or *A. subandinus* were used. The duration of the observation for each individual female was 10min or less when the female had attacked all established hosts. In contrast to experiment 2, females were not allowed to oviposit in their previously parasitised hosts during the first exposure time. The leaves were removed from the female after all three hosts were encountered. The same female was then introduced to the first leaf after 4hr, the second leaf after 8hr and the third leaf after 24hr. To examine conspecific-host discrimination of the parasitoids at different exposure times, an experiment similar to the first was conducted, but at the second exposure time (i.e. 4, 8 and 24hr after the first exposure) a conspecific female was used.

For both series of experiments, location of hosts was recorded on diagrams of the leaves (as described in Section 9.8.1, Fig. 9.1.A). The PTM larvae were dissected individually immediately after the last exposure time. The number of eggs and the approximate age of eggs (old or fresh egg) were also recorded on the diagram of each leaf.

The data from both sections were analysed with a Chi-square test, using Genstat 5 (Lane and Payne, 1996).

9.5.2 Results

The percentage of superparasitism by *O. lepidus* decreased with time for self and conspecific (Fig. 9.7). The results of data analysis indicated that there was a highly significant difference between the number of parasitised and superparasitised hosts for *O. lepidus* after 4hr, 8hr and 24hr from initial exposure time ($P< 0.001$).

Host discrimination by *O. lepidus* was improved with a greater interval between first and second exposures, and the parasitoid rejected parasitised hosts at 8hr and 24hr after initial parasitisation. Female *O. lepidus* were less likely to oviposit in hosts parasitised by themselves 4hr after initial parasitisation, whereas oviposition in conspecific parasitised hosts occurred to a greater extent (Fig. 9.9). The data indicated that both forms of host discrimination operate in *O. lepidus.*

A. subandinus females showed superparasitism on hosts parasitised by self and conspecifics up to 24hr (Fig. 9.8). The analysis of data showed no significant differences for *A. subandinus* at different intervals of exposure time ($P> 0.05$, Chi-square test).

9.5.3 Discussion

Females of both species laid more eggs in hosts parasitised by a conspecific wasp than in hosts parasitised by themselves. In female *O. lepidus*, when the host had been parasitised 4hr

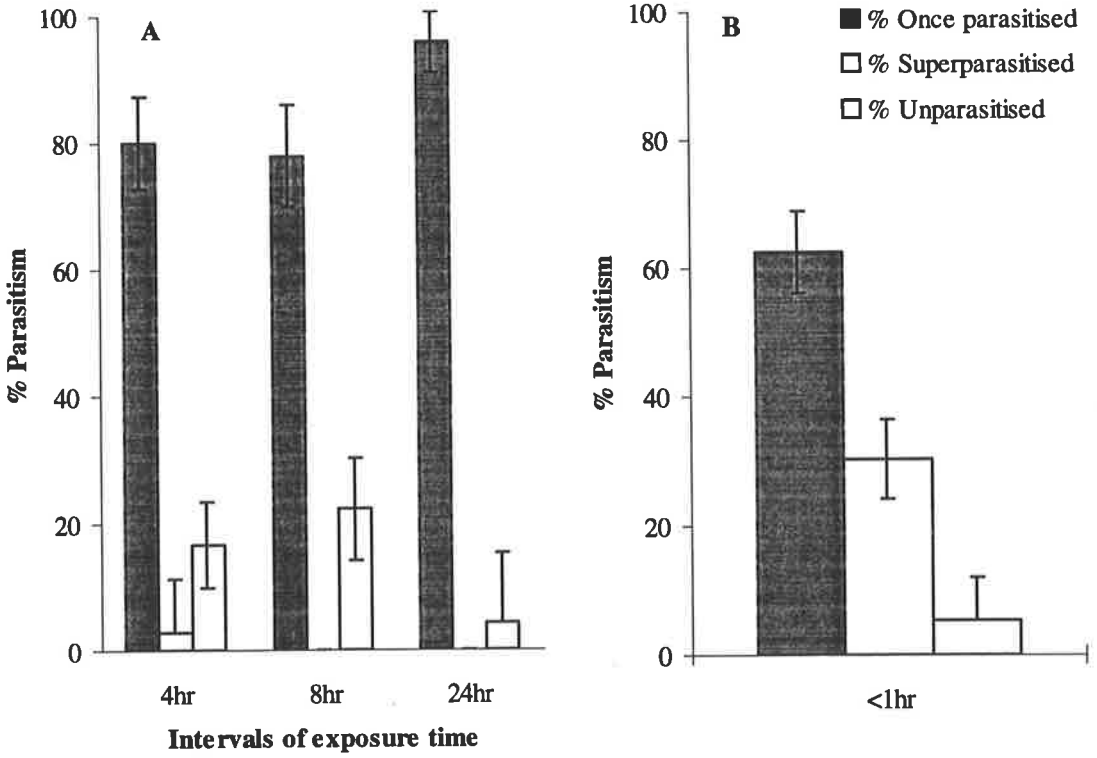
Figure 9.7: *O. lepidus* self and conspecific host discrimination at different intervals between exposure times. Comparison among 3 intervals of exposure time. Error bars represent standard error of mean.

A) self host discrimination at 4hr, 8hr, and 24hr in experiment 3 in Section 9.5.2 (n = 14),

B) self host discrimination at less than 1hr in experiment 2 in Section 9.5.2 (n = 20),

C) conspecific host discrimination at 4hr, 8hr, and 24hr in experiment 3 in Section 9.5.2 (n = 14),

D) conspecific host discrimination at less than 1hr in experiment 2 in Section 9.5.2(n = 20).


A
100
80
60
40
20
0
% Parasitism
4hr
8hr
24hr
Intervals of exposure time
B
% Once parasitised
% Superparasitised
% Unparasitised
<1hr

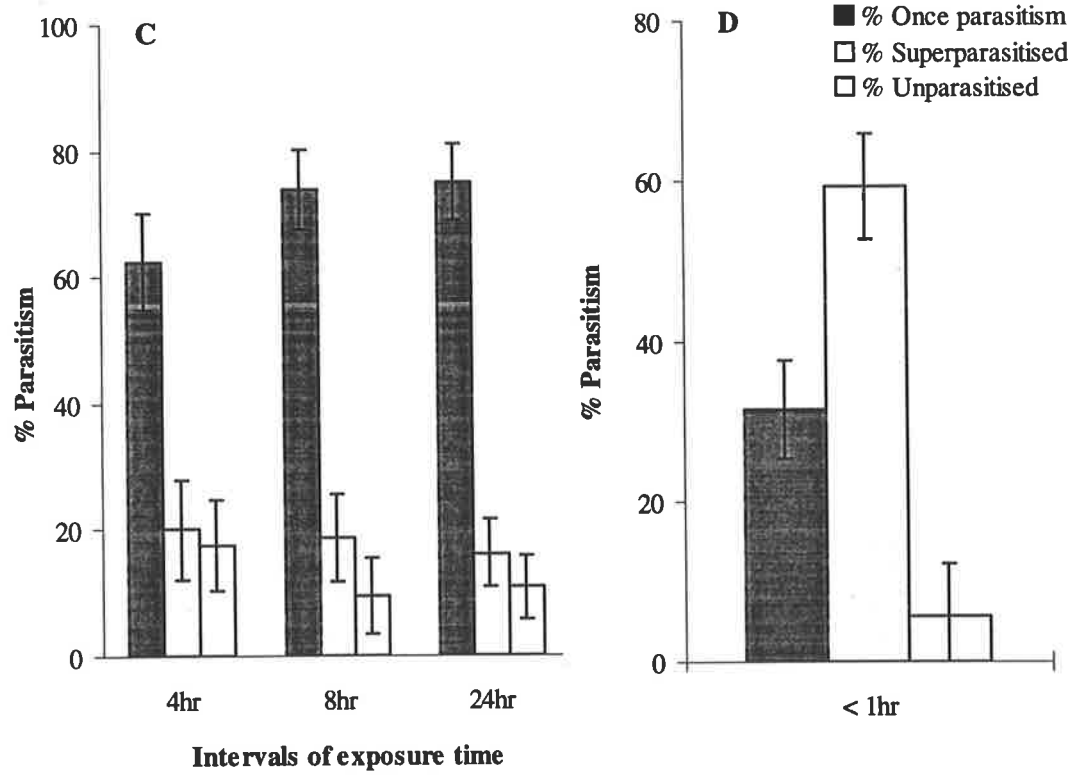

C
100
80
60
40
20
0
% Parasitism
4hr
8hr
24hr
Intervals of exposure time
D
% Once parasitism
% Superparasitised
% Unparasitised
< 1hr

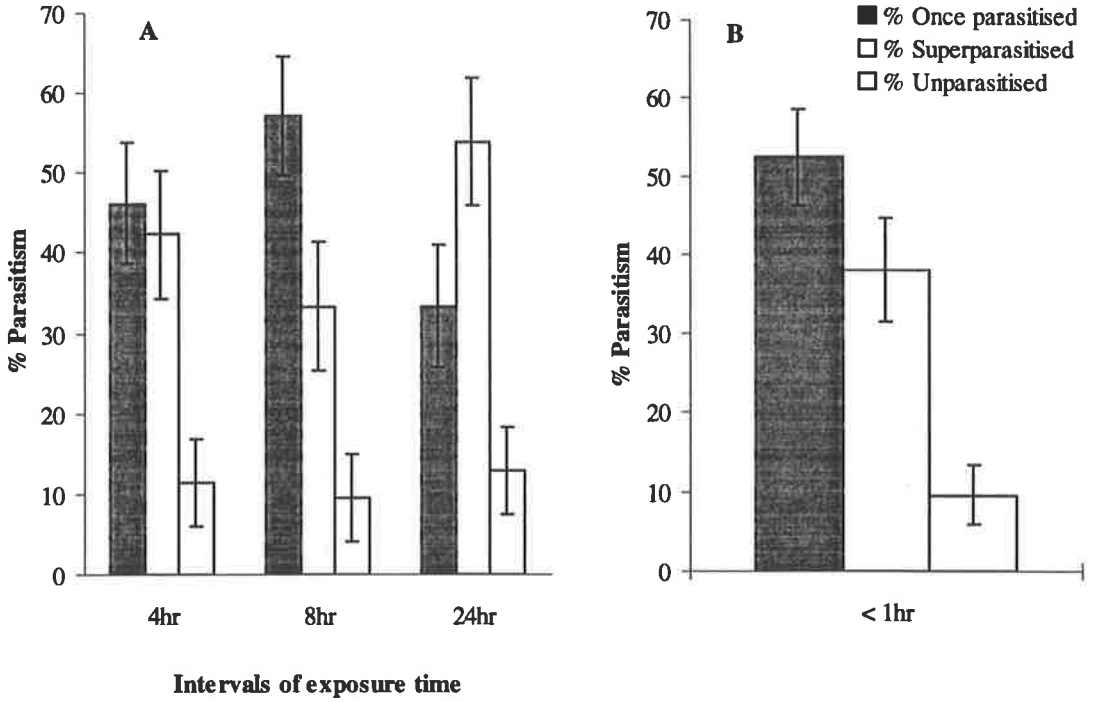
Figure 9.8: *A. subandinus* self and conspecific host discrimination at different intervals between exposure times. Comparison among 3 intervals of exposure time. Error bars represent standard error of mean:

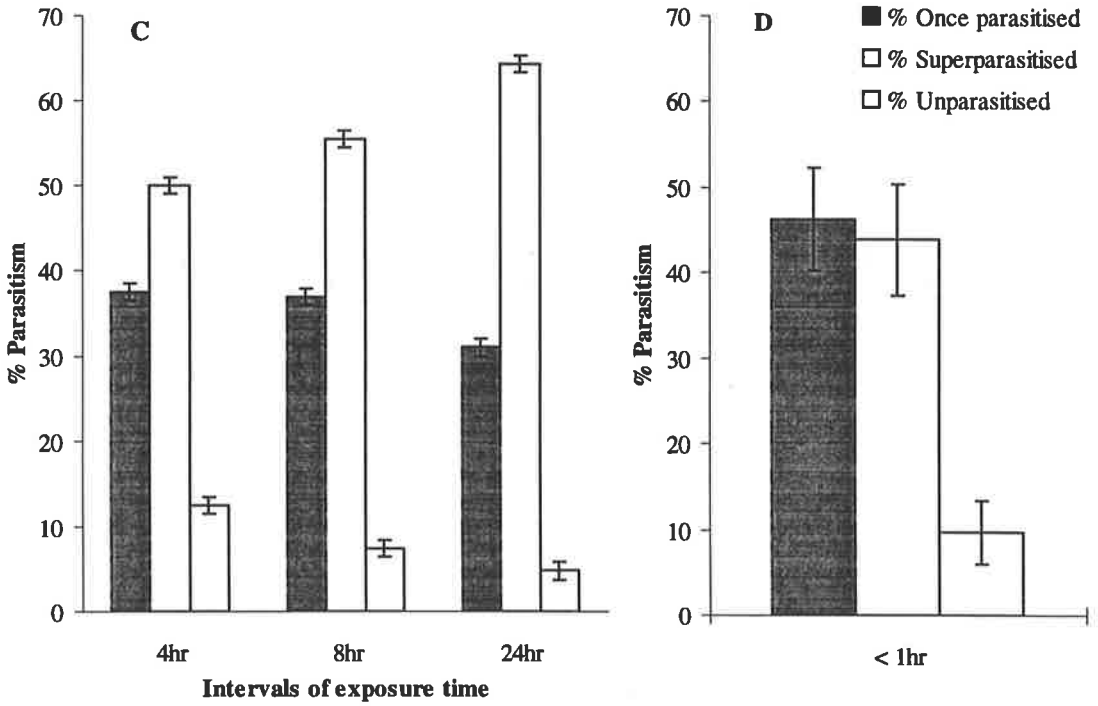
A) self host discrimination at 4hr, 8hr, and 24hr in experiment 3 in Section 9.5.2 (n = 15),

B) self host discrimination at less than 1hr in experiment 2 in Section 9.5.2 (n = 14),

C) conspecific host discrimination at 4hr, 8hr, and 24hr in experiment 3 in Section 9.5.2 (n = 15),

D) conspecific host discrimination at less than 1hr in experiment 2 in Section 9.5.2(n = 14).


A
B
% Once parasitised
% Superparasitised
% Unparasitised
% Parasitism
70
60
50
40
30
20
10
0
4hr
8hr
24hr
< 1hr
Intervals of exposure time


C
D
% Once parasitised
% Superparasitised
% Unparasitised
% Parasitism
70
60
50
40
30
20
10
0
4hr
8hr
24hr
< 1hr
Intervals of exposure time

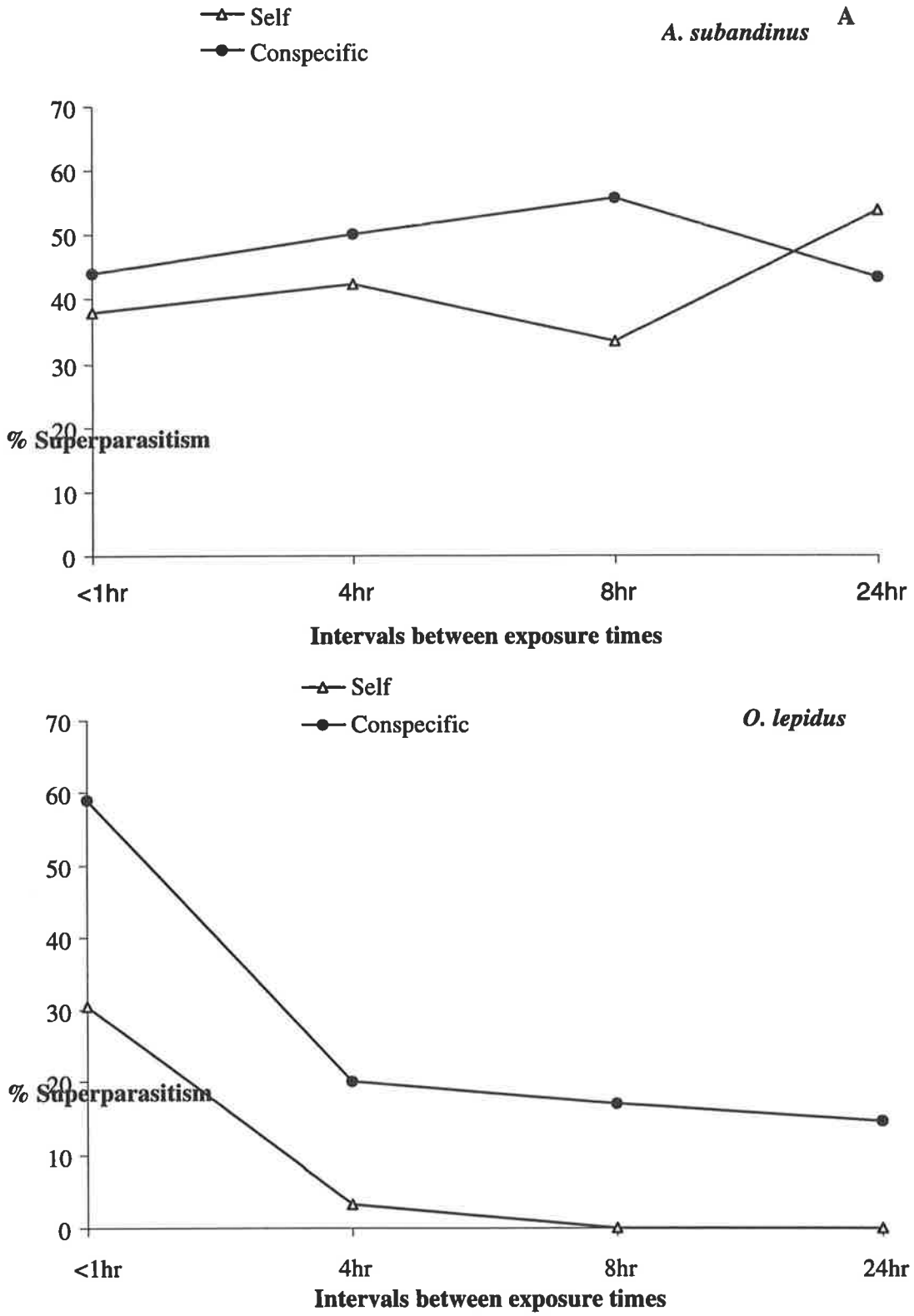


Figure 9.9: Comparison of % self and conspecific superparasitism by *A. subandinus* and *O. lepidus* at different intervals between exposure times. Hosts exposed at the longer intervals (4hr, 8hr and 24hr) were oviposited in once at the first introduction.

before, self and conspecific superparasitism was occasionally observed, but they never oviposited in a host which carried a 24hr-old embryo. In this species, hosts parasitised 4hr earlier were superparasitised by a conspecific female more than those parasitised 24hr before. Perhaps development of the parasitoid embryo influenced oviposition behaviour of subsequent females.

[IC3]It is possible that discrimination by female *O. lepidus* was related to (1) an increasing effect of pheromone marker left by females which Greany and Oatman (1972) analysed, (2) the developing parasitoid embryo of the first deposited egg making the host less acceptable than an unparasitised host, or (3) physiological changes in the host caused by parasitism.

It is suggested that host discrimination is time-dependent in *O. lepidus*, which is similar to some other parasitoids (Cloutier *et al.*, 1984; Chow and Mackauer, 1986). In *Aphidius nigripes*, an aphid parasitoid, the probability of superparasitism decreased as the interval between wasp exposure times increased up to 24hr after the initial attack (Cloutier *et al.*, 1984). This relationship was observed for *O. lepidus*, but not for *A. subandinus*. It seem there is no pheromone-like external mark left by the first-attacking female *A. subandinus* to influence acceptance of the second female.

9.6 Combination of host selection and time dependent host discrimination in *O. lepidus*

The aim of this experiment was to investigate the behaviour of female *O. lepidus* as they search on leaves infested with both a host parasitised once by self or a conspecific and an unparasitised host. The interval of time between first and second exposure to the hosts was varied to determine how this influences the choice of parasitoids.

9.6.1 Materials and methods

In this experiment, female *O. lepidus* were allowed to search and oviposit on only one of two PTM larvae in the first exposure time. In the second exposure, the wasp was given a choice between the unparasitised and parasitised PTM larvae and it was free to oviposit in any host. Each leaf was presented to a wasp twice. The second exposure followed < 1hr or 4hr after initial exposure. All possible combinations of two female wasps in sequence for the two intervals was used in each replicate. Oviposition behaviour were recorded with the Observer Version 2 (Noldus, 1991).

The observation was terminated after the wasp encountered both parasitised and unparasitised larvae. The first and second stages of the experiment examined females' time dependent host discrimination and the second stage examined their choice and ability to discriminate between parasitised and unparasitised hosts.

Twenty six replications were conducted in this experiment and data were analysed in a Chi-square test using SAS (SAS Institute, 1995).

9.6.2 Results

There were highly significant differences between the total parasitised and superparasitised hosts in each treatment. Heterogeneity tests indicated that there were no differences due to the two exposure intervals in the relative frequencies of once parasitised and superparasitised hosts with either the self superparasitised (2-tailed Fisher exact test, P= 0.687) or conspecific superparasitism (P= 1.00) categories. When data from the two time intervals were pooled, it was found that there was a greater incidence of conspecific superparasitism than self superparasitism (χ^2 with continuity adjustment = 5.949, df= 1, P= 0.015) (Table 9.2). This indicates a higher self-host discrimination ability of female *O. lepidus* than conspecific-host discrimination of this parasitoid (Fig. 9.10).

Table 9.5: Contingency table of categories of parasitism when females *O. lepidus* were introduced to previously parasitised hosts by self or conspecific at <1hr or 4hr intervals in a choice experiment (Chi-square test, using Genstat 5).

Treatments	*P*	χ^2	df
Overall comparison of 3 treatments	0.06	7.25	3
<1hr Self vs < 1hr conspecific	0.07	3.22	1
<1hr Self vs 4hr Self	0.05	0.34	1
<1hr Self vs 4hr conspecific	0.11	2.56	1
<1hr conspecific vs < 4hr Self	0.03	4.64	1
<1hr conspecific vs 4hr conspecific	0.90	0.01	1
4hr Self vs 4hr conspecific	0.04	3.96	1

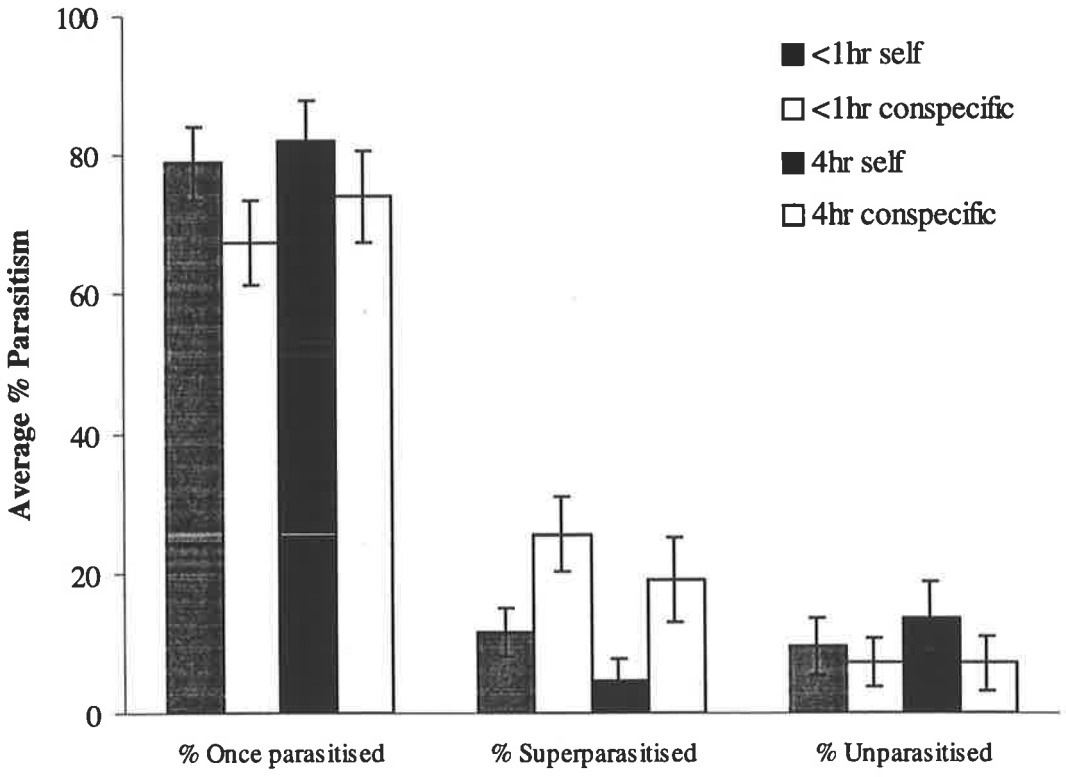


Figure 9.10: The choice made by *O. lepidus* females between unparasitised hosts and hosts parasitised by self and conspecific at less than 1hr and at 4hr after first exposure time. Error bars represent standard error of mean. Chi-square test indicated that there was significant differences between once parasitised and superparasitised hosts in self and conspecific at both the exposure <1hr and 4hr intervals ($P< 0.05$).

9.6.3 Discussion

Comparison of the different treatments in this experiment (Table 9.2) with previous results obtained for *O. lepidus* (Sections 9.4.2 and 9.5.2) confirmed that this parasitoid was able to discriminate self-parasitised hosts after 1hr. In addition, the presence of an unparasitised host in the vicinity of a parasitised host does not effect discrimination by females. The results confirmed conclusions from previous experiments on self and conspecific host discrimination by *O. lepidus*. The trend of decreasing superparasitism after 1hr for self and after 4hr for conspecific superparasitism is similar to the previous experiment (Sections 9.5.2).

9.7 Interspecific host discrimination in *A. subandinus* and *O. lepidus*

[IC4]The ability of a female parasitoid to recognise a host previously parasitised by another species is termed interspecific (heterospecific) host discrimination. When females of different species lay one or more eggs in a parasitised host it is known as multiparasitism (Doutt and DeBach, 1964; Strand, 1986). The inability of the two parasitoids to distinguish between unparasitised hosts and hosts parasitised by another parasitoid species may be a reason for their failure to coexist on their host (Pijls *et al.*, 1995).

This experiment was carried out to determine whether females of the two parasitoid species discriminate between unparasitised hosts and hosts previously parasitised by an individual of the other species 24hr after the initial oviposition.

9.7.1 Materials and methods

The procedures for this experiment were similar to experiment 3 (Section 9.5.1). In this experiment one female of one species was released into the wind tunnel in which a potato leaf with three established unparasitised larvae had been placed. After the first female encountered the three larvae, she was removed and the leaf kept in an empty cage for 24hr.

The second female of the other species was introduced to the plant with hosts parasitised 24hr earlier by an individual of the first parasitoid species.

Each female was allowed to search on leaves for 10min. The host selection behaviour of parasitoid females was recorded in the same conditions used for previous experiments in the wind tunnel. Apparent oviposition by parasitoid females was recorded on a diagram of each infested plant (Fig. 9.1.A).

This experiment was repeated 26 times under the same conditions. In 13 replicates, females of *A. subandinus* were released individually first, with *O. lepidus* being released 24hr later, and in the other 13 replicates the sequence was reversed. The larvae were collected after each test and dissected to determine the rate of parasitism.

Data were analysed with a Chi-square test using Genstat 5 (Lane and Payne, 1996) to compare the number of parasitised, superparasitised and multiparasitised hosts in each treatment.

9.7.2 Results

In this choice experiment female *A. subandinus* accepted hosts previously parasitised by *O. lepidus* and vice versa (multiparasitism). There was a high percentage of multiparasitism in both combinations (i.e. *Apanteles* first + *Orgilus* second and *Orgilus* first + *Apanteles* second) (Fig. 9.11).

The first analysis of data compared the distribution of parasitism categories for the two experiments. The results indicate that the distributions did not differ between the experiments (χ^2 = 4.94, df= 5, *P*= 0.551). In the second analysis, the distribution of eggs laid by the second female was examined. If hosts were parasitised randomly, then it would be expected the frequency of once parasitised and multiparasitsed hosts would be in the same proportion as unparasitised and parasitised hosts, respectively, left by the first female. The analysis showed

that there were fewer multiparasitised hosts than would be expected. This may indicate an avoidance of multiparasitism or reduced attractiveness of parasitised hosts. The relatively high frequency of multiparasitism (greater than half that expected by chance) suggested that wasps did not avoid multiparasitism (Table 9.3). Females of both parasitoids responded positively to hosts parasitised by each other with antennal drumming and ovipositor insertion.

Table 9.6: Contingency table of categories of parasitism when wasps were exposed to previously parasitised hosts by interspecific (Chi-square test, using Genstat 5).

Order	**1st species: 1st visit**		**2nd species: 2nd visit**	
	Observed values			
	Parasitised	Unparasitised	Once- and super-parasitised	Multiparasitised
O. lepidus / A. subandinus	68	68	33	22
A. subandinus / O. lepidus	95	66	57	38
Assuming all hosts are susceptible to parasitism			Expected values	
			Once- and super-parasitised	Multiparasitised
O. lepidus / A. subandinus			19.7	35.3
A. subandinus / O. lepidus			38.9	56.1
Chi-squared probability of this result:				1.13E-07
Assuming unparasitised hosts are immune to parasitism because they are inaccessible (e.g. those in veins)			Expected values	
			Once- and super-parasitised	Multiparasitised
O. lepidus / A. subandinus			18.0	37.0
A. subandinus / O. lepidus			36.4	58.6
Chi-squared probability of this result:				**8.78E-10**

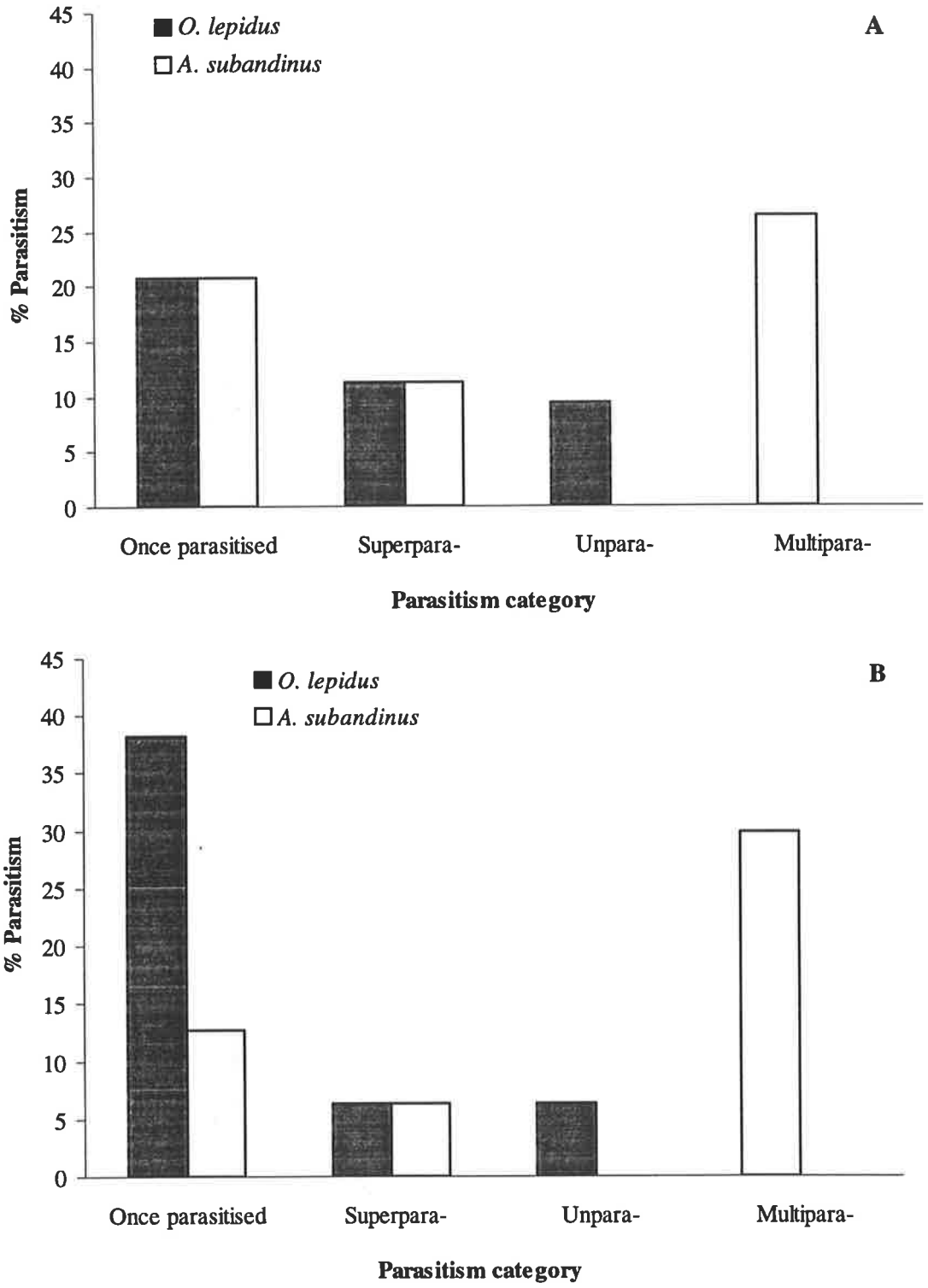


Figure 9.11: The percentage of parasitism when: **A)** females of *A. subandinus* were released first, followed by *O. lepidus* 24hr later and **B)** vice versa (n = 26 females and 78 hosts).

9.7.3 Discussion

In both species, females did not discriminate interspecifically parasitised hosts. They accepted a high percentage of hosts parasitised 24hr previously by each other. Although females of *O. lepidus* were capable of time-dependent self and intraspecific host discrimination (Section 9.5.2), a high degree of multiparasitism occurred in hosts previously parasitised by *A. subandinus*. Thus it seems that the cues used by these parasitoids are specific. It is suggested that multiparasitism is adaptive for both parasitoid species. However, many parasitoids discriminate hosts parasitised by other species after 2 days (e.g. see Tillman and Powell, 1992a&b).

This information gives an insight into the development of release strategies to improve the coexistence and develop biological control with these species. The presence of once parasitised hosts for both species indicates their complementary nature in biocontrol of PTM.

9.8 Comparison of the ovipositional behaviour of female parasitoids and the rate of parasitism at different host locations in the mined leaf

In this section, the host discrimination behaviour of the two parasitoids was compared in different experiments. By recording data with the Observer and other information on the diagram of the infested leaves during each test, the relationships between host location, length of attack and superparasitism were investigated.

Observations suggested that host discrimination by female parasitoids was different with varying locations of the host inside the mines on infested potato plants. To investigate this hypothesis, data recorded from previous experiments and leaf diagrams were used that showed the location of parasitised and superparasitised hosts on the leaves.

The aim was to find answers to the following questions:

1) Do females of *O. lepidus* and of *A. subandinus* lay eggs by inserting the whole length of their ovipositors inside the mines?

2) How many eggs do they lay in each attack?

3) Does the duration of their ovipositional behaviour change when they encounter unparasitised and parasitised hosts?

4) Do their discrimination abilities change with a changing location of hosts inside the leaf tissues?

9.8.1 Materials and methods

The three common oviposition behaviours of female wasps recorded within a limited time for each female in previous experiments were compared. The behaviours of the wasps were categorised as antennating, shallow probing and deep probing (Section 9.4.1). Furthermore, the periods of searching behaviour of the female wasps and the percentage of parasitised, superparasitised and unparasitised hosts were compared when they were introduced to foliage with different mining damage. In order to find a reliable behavioural criterion which verified that an egg was actually laid, additional behavioural observation was needed. Therefore, by observing, recording and immediately dissecting hosts attacked by parasitoids, the position of wasps when laying an egg was clarified.

Individual wasps were observed continuously for 10 to 20min (in different experiments), after the parasitoids landed on the plant. In these experiments the length of the searching period between the two ovipositions or internal time (Van Lenteren, 1991) was not recorded.

To compare the duration of the above behaviours when the host was in different parts of the plant tissues (i.e. mesophyll, vein or petiole), a diagram was made of each leaf to distinguish

host locations (Fig. 9.1.A). Larval activity traces were also described on the leaf diagram (e.g. the depth of larval activity or the length of tunnel dug by the larva in the veins).

The periods of searching behaviours of the wasps were compared when they parasitised hosts once or superparasitism occurred in the second exposure, using t-test in Genstat 5 (Lane and Payne, 1996).

9.8.2 Results

a) Oviposition behaviour

In general, the mean duration of the three searching and ovipositing behaviours for *A. subandinus* females (14.5s) was less than that of *O. lepidus* females (28.6s) (Table 9.4). Direct observation indicated that *A. subandinus* searched faster than *O. lepidus*. The duration of deep probing was longer than antennating and shallow probing for both *A. subandinus* and *O. lepidus.*

Table 9.7: Comparison of the mean duration of the three searching and ovipositing behaviours of *A. subandinus* and *O. lepidus* females. Time in second (s), and sem in the brackets.

Parasitoid	**Antennating**			**Shallow probing**			**Deep probing**		
	s	n	%	s	n	%	s	n	%
A. subandinus	3.1 (0.34)	2684	21.4	8.1 (0.82)	2432	55.9	3.3 (0.24)	644	22.8
O. lepidus	6.7 (0.48)	1043	23.5	16.4 (0.82)	992	57.3	5.5 (0.23)	428	19.2

The period of antennating and deep probing did not show significant differences when wasps encountered hosts the first or second time, to once parasitised or superparasitised and hosts mined in the vein or in mesophyll of leaves (P> 0.05, t-test). The duration of shallow probing

was not significant different for self parasitised host whereas, there was significantly differences for conspecific parasitised host or when host located in mesophyll or vein ($P<0.05$, ANOVA). The shallow probing lasted longer for larvae located in veins and petioles, than for those mined inside the mesophyll (Fig. 9.12). This confirmed the results of the direct observations that showed number of encountering with the larvae mined inside the wider or longer mines was more than those inside a small mine in the mesophyll.

Oviposition by parasitoids in deep probed larvae was confirmed by dissection of the hosts. In some cases, females of both species inserted of the whole of their ovipositor inside the plant, but did not always lay an egg. It appeared that in the ovipositing position, the female *O. lepidus* remained motionless while the egg was being laid, while in deep probing behaviour where an egg was not laid, the wasp was continuously shaking her abdomen. Furthermore, after oviposition, the female left the site immediately. Dissection of parasitised larvae (Tables 9.5 and 9.6), did not show a distinctive puncture mark to be made by the penetration of the parasitoids ovipositor.

b) Superparasitism

The percentage of superparasitism by the two parasitoids increased when hosts mined a long tunnel in the veins and crawled into the thick tissues of plant. In these situations, the ability of the female wasp to find and parasitise hosts within the plant tissues was limited by the length of their ovipositors. Larvae of PTM were patchily distributed amongst the mesophyll, the newly established larva generally makes a blotch mine in the mesophyll, but later (24hr), works down and penetrates a tunnel inside the vein, petiole and stem. Observations of PTM larvae on the leaves indicated that more than 77 % (in all experiments in this chapter) of the established larvae crawled in the veins of leaves after 24hr. The PTM larvae seemed to sense

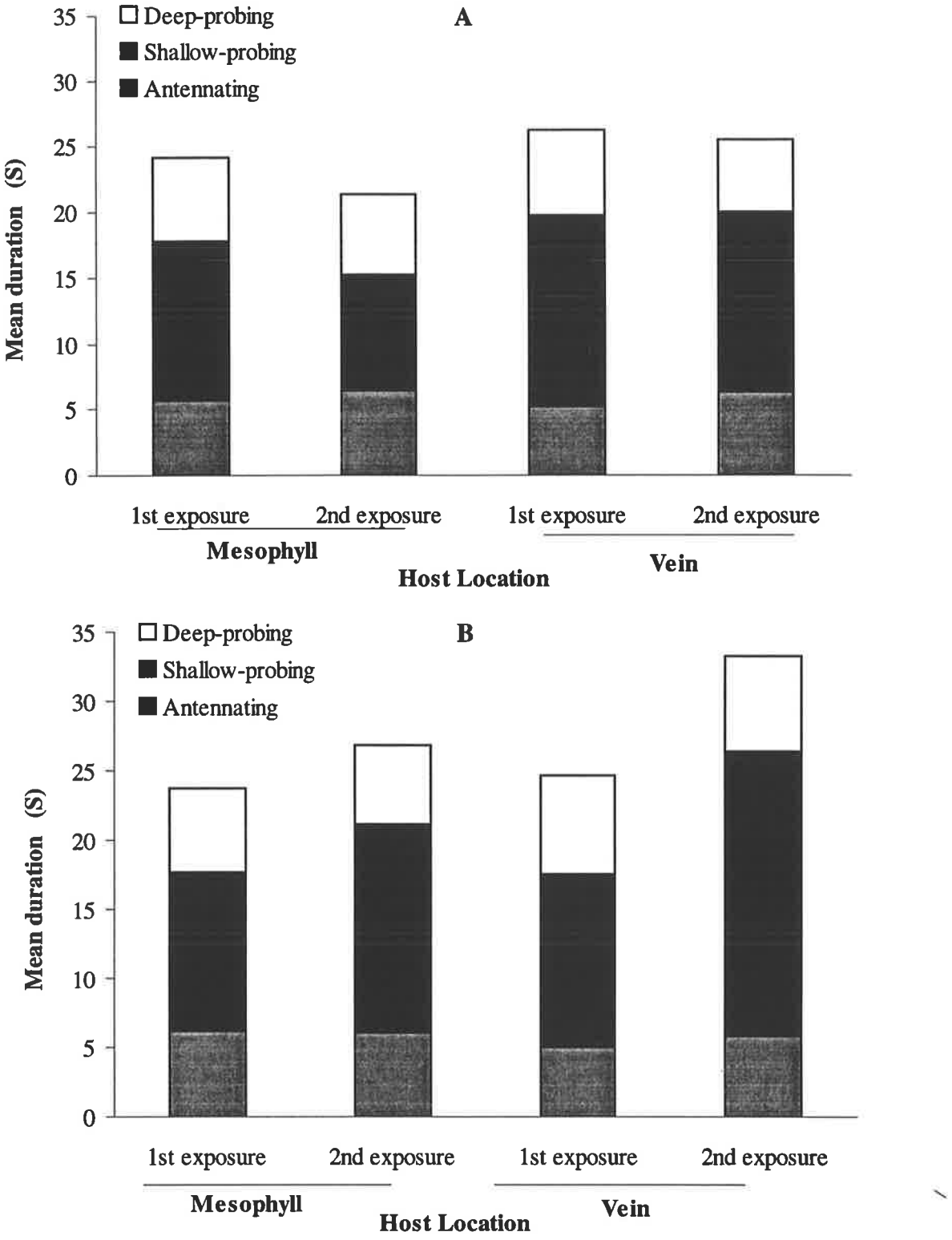


Figure 9.12: The mean duration of 3 oviposition behaviours of *O. lepidus* females at two exposures to self and conspecific parasitised hosts inside the mesophyll and veins. **A)** Self, **B)** Conspecific.

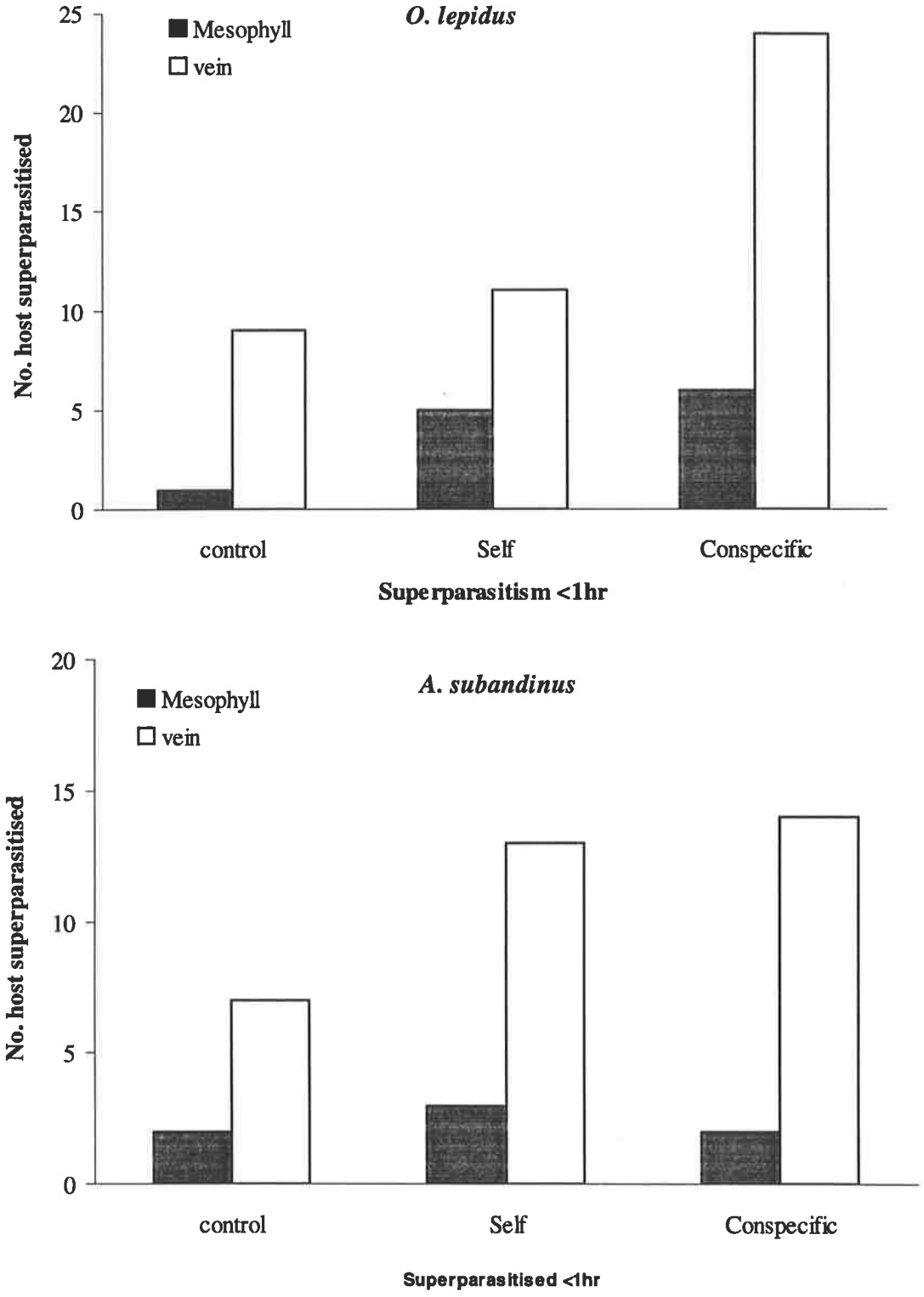


Figure 9.13: The number of superparasitised hosts by *O. lepidus* and *A. subandinus* on hosts mined inside the mesophyll and vein of potato leaves.

the presence of the parasitoids and quickly retreated along its tunnel until it was out of reach of the parasitoid.

Female *A. subandinus* followed the crawling larva inside its tunnel and inserted her ovipositor from different locations of tunnel mined by the host. The female then moved rapidly and frequently rotated herself about the axis of the inserted ovipositor while rapidly palpating the plant surface with her antennae. This behaviour confirms that females can parasitise hosts inside the long tunnel with her short ovipositor (0.63mm). In comparison with *A. subandinus*, female *O. lepidus* inserted her long ovipositor (4.5mm) from one side of the tunnel mined by the host (not usually the mine entrance that was covered with host faeces) and turned it in trying to touch the host.

O. lepidus and *A. subandinus* females had difficulty in discriminating self- and conspecific- and interspecific-parasitised hosts which had burrowed long tunnels inside the plant tissues and the rate of superparasitism in this situation was higher than when the host was located in a small blotch (Fig. 9.13).

9.8.3 Discussion

Comparison of the results of the parasitism obtained from different experiments in this chapter and also observations on the searching and oviposition behaviour of *A. subandinus* and *O. lepidus* indicated that both species can oviposit in hosts mined inside the plant tissues. However, the duration of oviposition behaviour and rate of superparasitism increased when hosts were located in the longer and wider mines. The rate of superparasitism by the two species is related to location of host and the extent of its feeding activity in the mine.

From these observations it can be implied that an attack by *A. subandinus* or *O. lepidus* was a good indication of oviposition, except where hosts made a long tunnel inside the leaf veins or petiole. Although the female wasp can find the host location by walking and antennating on

both surfaces of the leaf, the duration of a female probing a host and oviposition were absolutely dependant on the location of PTM larvae. The number of ovipositor insertions by *A. subandinus* and *O. lepidus* females was significantly higher when the host was inside the thick plant tissue (veins or petioles) than those located in the mesophyll ($P< 0.05$).

A. subandinus and *O. lepidus* females could insert their ovipositor into the mine through the plant epidermis, but they frequently had difficulty in locating a host. Females followed a PTM larva during its movement inside the mine and repeatedly inserted the length of their ovipositor from lower and upper surfaces of the leaf. Observations by other researchers on the leaf mining insects suggested that vibration signals produced by larvae are a source of information to foraging parasitoids (Meyhofer *et al.*, 1994). It is possible that *A. subandinus* females follow vibrational signals produced by a moving larva. Older PTM larvae had longer and wider tunnels in veins and other thick plant tissues, and it is assumed that this is the reason why 1-3-day-old PTM larvae are a suitable host stage for higher parasitism by the two species (Cardona and Oatman, 1975; Oatman *et al.*, 1969).

A. subandinus and *O. lepidus* females probed hosts with their ovipositors many times without oviposition. In addition, only one egg was laid in each encounter; this was confirmed by dissecting many oviposited larvae which mined inside the mesophyll and were only encountered once. Furthermore, it seems these parasitoids are similar to *Antrocephalus pandens* Walker which detects parasitised hosts using sense organs on the ovipositor (Gates, 1993).

In general, *A. subandinus* and *O. lepidus* females attach one egg to the internal organs of PTM larvae (Section 8.4.2) in one complete oviposition attack (deep probing with motionless behaviours). Their egg laying is not related to the insertion of the whole length of the ovipositor inside the mine, but it depended on depth of the mine and host accessibility. The

number of encounters with hosts is greater and the duration of oviposition behaviours of wasps is longer when hosts mine inside the thick tissue of plants. Finally, the host discrimination ability of both species is reduced when hosts are located inside deep mines compared to hosts that are feeding in a small blotch.

9.9 General results

Superparasitism by *A. subandinus* and *O. lepidus* was observed in all tests when parasitised hosts were introduced immediately after the first oviposition by the female wasps. In the case of superparasitism, no more than 6 eggs were found in a parasitised larvae. When females of both parasitoid species were allowed to encounter and attack all available PTM larvae on the potato plants, a mean of 9% of the hosts escaped parasitism, all of which mined in the veins or petioles of leaves. There were no significant differences between two species in this regard (Tables 9.3 and 9.4).

Table 9.8: Percentages of superparasitised and unparasitised hosts in different experiments of host discrimination by *O. lepidus*.

Experiment	Host location	no. superparasitised	no. Unparasitised	Total host
9.4	mesophyll	12	0	210
	vein	44	22	
9.5	mesophyll	2	0	187
	vein	22	22	
9.6	mesophyll	5	0	193
	vein	25	18	
9.7	mesophyll	2	0	161
	vein	50	7	
Total		162	69	751

From 162 (21.5%) hosts superparasitised by *O. lepidus* in all experiments, 87% were recorded inside the vein and thick tissues and the remainder (13%) mined inside the mesophyll. This rate for *A. subandinus* was 80% and 20% respectively in the vein and mesophyll.

Table 9.9: Percentages of superparasitised and unparasitised hosts in different experiments of host discrimination by *A. subandinus*.

Experiment	Host location	no. superparasitised	no. Unparasitised	Total host
9.4	mesophyll	7	0	125
	vein	34	13	
9.5	mesophyll	7	0	179
	vein	32	17	
9.7	mesophyll	8	0	106
	vein	20	5	
Total		108	35	410

There were 2-3% missing or dead larvae in all of the observations in different experiments with the two parasitoids. There was no oviposition in these hosts, because no probing was recorded by the observer after antennating. Thus, females of both species appeared to be stimulated by a moving host and accepted it for oviposition.

9.10 General discussion

In these laboratory experiments, when *O. lepidus* females were given a choice between infested potato plants with parasitised and unparasitised PTM larvae, they selectively flew to the plants with unparasitised hosts. *A. subandinus* females lack this ability and could not distinguish plants with unparasitised hosts from those that had previously parasitised hosts.

In comparisons between parasitised and unparasitised hosts, host discrimination by both species was observed less than 1hr after the first oviposition. However, *A. subandinus* had a greater ability of host discrimination than *O. lepidus* at less than one hour. *O. lepidus* rejected parasitised hosts more often when the interval between host encounters increased, and wasps never accepted a host parasitised 24hr before by a themself. However, there was a similar rate of superparasitism by a conspecific from 4hr to 24hr after the first parasitism (Fig. 9.9). These results concur with previous investigations by Greany and Oatman (1972), who reported that *O. lepidus* could discriminate parasitised hosts, but their study did not reveal the means by which the parasitoid detects the condition of the host. *A. subandinus* females demonstrated an avoidance of self and conspecific superparasitism when the host was parasitised less than 1hr previously and this ability decreased gradually. However, in the presence of a 24hr-old embryo the percentages of superparasitism by females were often low, particularly on hosts previously parasitised by a conspecific. Both species have multiparasitisation under particular conditions as described previously. Females of neither species discriminated between hosts parasitised by each other up to 24hr.

It seems that exposure time influences the rate of superparasitism by the two species. *O. lepidus* females showed a lower percent superparasitism of self and conspecific after a short exposure time than when they were exposed to a given number of hosts for a longer period (Section 6.2.2). That is, the percentage of superparasitism increased as time of host exposure to the parasitoid increased. This is due to wasps encountering more and more unsuitable hosts as time progressed (i.e. hosts mined inside the veins or other thick tissue) and superparasitism occurred mostly on these unsuitable hosts (Tables 9.8 and 9.9).

One mechanism of host discrimination by *O. lepidus* females was found by Greany and Oatman (1972). They found that wasps leave a pheromone that deters superparasitisation. The development of embryos, in addition to changes in host quality (Fisher and

Ganesalingam, 1970; Vinson, 1976) are possibly the other factors involved in host discrimination by *O. lepidus*. It is possible that *A. subandinus* females use a combination of these factors in the host discrimination process.

Many researchers have found that host discrimination occurs in response to external or internal markers (e.g. Fisher and Ganesalingam, 1970; Rogers, 1972; Vinson, 1976; Podoler and Mendel, 1977; Chow and Mackauer, 1986; Hubbard *et al.*, 1987; Gates, 1993). Van Lenteren (1976, 1981) described four categories of factors as possibilities for intraspecific or interspecific recognition of parasitised hosts: 1) physical changes in the host, 2) changes in the haemolymph of the host, 3) substances left by the female parasitoid as a source of cues and 4) substances originating from the egg or larva of the parasitoid in a parasitised host.

In conclusion, *O. lepidus* females discriminate between parasitised and unparasitised PTM larvae under a broad range of conditions such as exposure time (distance between first and second encounter) and host location (suitable or unsuitable host). Results obtained from experiments 2, 3 and 4 (Sections 9.4.2, 9.5.2 and 9.6.2) are evidence for a highly-developed ability to avoid self-superparisitism (i.e. self-discrimination) by *O. lepidus*. Self-superparasitism is relatively less profitable than conspecific superparasitism under most ecological conditions (Van Lenteren *et al.*, 1978; Van Lenteren, 1981; Van Alphen and Visser, 1990; Visser, 1993). However, *O. lepidus* is one of only a few species for which self discrimination has been demonstrated (Van Alphen and Visser, 1990; Visser, 1993; Ueno and Tanaka, 1996). The data presented in this chapter confirmed the results of Greany and Oatman (1972) concerning host discrimination. The observed self-discrimination indicates that *O. lepidus* leaves an individual-specific mark on hosts following oviposition. It is most economical for this species to parasitise all hosts once and then leave the patch. Self discrimination was shown to be imperfect, especially when hosts mined leaf veins and petioles.

A. subandinus females laid few eggs in either the self or conspecific treatments, indicating host discrimination in both cases at less than 1hr period between first and second exposure. Females of both species showed multiparasitism even 24hr after the first oviposition by an other species. There was a highly significant difference between the number of hosts superparasitised inside the long tunnels mined in the veins, or thick tissues of the plant with those superparasitised inside the mesophyll. In addition, the oviposition behaviour of both parasitoid females lasted longer when they encountered an unparasitised host than when they encountered a host that was previously parasitised. It is likely that *O. lepidus* and *A. subandinus* females use a combination of factors such as pheromone marking and physiological changes in the embryo inside a host to discriminate previously parasitised hosts.

Chapter Ten

General discussion and conclusions

"I keep six honest serving-men, they taught me all I knew. Their names are What and Why and When and How and Where and Who." R. Kipling, 1895

10.1 General discussion

In spite of the world-wide importance of PTM, little has been published about the factors influencing the efficiency of its two most important parasitoids, *Apanteles subandinus* Blanchard and *Orgilus lepidus* Muesebeck. These parasitoids have been released in Australia and have established in all potato growing regions. However, attempts to control PTM populations with these parasitoids have only been partially successful. Could both biotic and abiotic factors have contributed to the incomplete effectiveness of the two parasitoids?

In this project, a series of experiments were conducted to provide information on reproductive ability, responses to temperature, searching behaviour, density dependence, functional response to host density, competition and host discrimination of the two species. What follows is a summary of the results. This information will help to maximise the potential of these parasitoids as biological control agents of PTM in inundative releases.

The developmental times of the two parasitoids and their host were studied using strains collected from South Australia and Victoria. The development of both parasitoids was faster than their host. It is advantageous for parasitoids of PTM to be synchronised to the pest population. *A. subandinus* has a shorter developmental time than *O. lepidus* particularly at the egg and larval stages. This is also advantageous for the two species to coexist in their area of activity (Section 3.4). The results of the laboratory experiments and the field survey conducted in 1995-1997 revealed that temperature has a profound influence on the development and number of generations of both the parasitoids and their host. Temperature also effects the longevity of the two parasitoids. At very high temperatures they have a short life span and may not live long enough to have an effect on their host in terms of rate of parasitism.

Data obtained in the laboratory supported the results of field monitoring in that *A. subandinus* was in greater numbers than *O. lepidus* in cool areas (Section 4.5.2). These results reinforced previous investigations by Horne (1990) who reported that *A. subandinus* developed larger populations than *O. lepidus* early in the growing season which is attributable to a developmental advantage in cool conditions. My analysis of temperature-dependent development indicated that *A. subandinus* is better adapted to cooler climates than *O. lepidus*. This study explains in part, the findings of Briese (1980) that the abundance of these parasitoids depends on their adaptation to different climatic conditions and that *O. lepidus* is more abundant in warm climates.

The study of reproductive activity showed that oviposition by the two species depends on both host density and temperature. However, female *O. lepidus* has the advantage of a higher capacity for egg production than female *A. subandinus*. The results of wasp dissections and experiments on their reproductive ability showed that female *O. lepidus* produces a maximum of 275 eggs and *A. subandinus* 215 eggs in laboratory conditions. Even under optimal conditions of temperature, and food and host availability these eggs could not all be utilised. Thus, Australian strains of *O. lepidus* oviposit in a maximum of 243 hosts and *A. subandinus* 199 hosts during their lifetime in optimal conditions (Tables 4.3 and 4.4). The results of the development and fecundity studies of the two species suggest local adaptation, as the results differed substantially from those reported for these species in America (Oatman *et al.*, 1969; Cardona and Oatman, 1975).

Two problems were found in studies of the reproduction of *A. subandinus* in the laboratory. One was a high level of superparasitism, and the other was the increasing proportion of males in the culture, which was possibly related to inbreeding after a few generations in the laboratory. These problems were not observed in *O. lepidus*. In addition, high host numbers should be provided (at least 50 established larvae per female per day) to reduce

superparasitism. Further, wasps should be replaced with newly collected individuals from the field after 3-4 generations to reduce inbreeding and decrease the ratio of males in the culture. Alternatively, large cultures could minimise inbreeding.

Volatile chemicals associated with host feeding on plants attract both *A. subandinus* and *O. lepidus* to the host (Chapter 5). These results could be useful as basic information for future work to develop traps to attract parasitoids in the field and perhaps to enhance the rate of parasitism on infested crops.

Experiments in the field and laboratory in which *A. subandinus* and *O. lepidus* searched in patches of plants with a range of host densities indicated conditional density dependency of these parasitoids. Percent parasitism by *A. subandinus* increased with increasing host density in both laboratory and field experiments, except when the range of host densities was small in all patches and plants were small. In contrast to *A. subandinus*, females of *O. lepidus* showed a density dependent response in the field when provided with a wider range of host densities. The percent parasitism of this parasitoid under laboratory conditions with a small range of host densities and small plants showed a density independent characteristic.

The results obtained from the reproduction studies of both species in the laboratory indicated that most hosts are susceptible to parasitism in potato foliage. However, a few that inhabit the petioles and large veins may avoid parasitism. When hosts are limited, superparasitism occurs and increases over time. Nevertheless, both avoide self-, con- and inter-specific superparasitism.

The functional responses of both *O. lepidus* and *A. subandinus* were best approximated by a Type 2 curve (Holling, 1959; Hassell, 1978). However, in neither case was an upper asymptote observed indicating that handling time is not a significant factor in their functional

responses. The functional response of *A. subandinus* was shown to be affected by temperature and it is likely that the behaviour of *O. lepidus* is similarly affected.

During this study, it was found that one of the important factors affecting the oviposition rate of the parasitoids, and causing an increase in superparasitism, was unsuitable host locations. Hosts located in long or wide mines were less accessible to parasitoids. Of those that were parasitised the majority were superparasitised. This is due to a reduced ability of parasitoids to discriminate parasitised hosts in such mines.

The available information indicates that *O. lepidus* is superior to *A. subandinus* in both reproductive potential and larval competitive ability. *O. lepidus* oviposits in more hosts than *A. subandinus* when exposed to hosts simultaneously, but despite its superiority, it is not as effective at extremes of temperature. The results of this study can be summarised in the following points:

1. Fecundity and oviposition by female *O. lepidus* was higher than *A. subandinus*,

2. responses of female *O. lepidus* and *A. subandinus* varied with varying host density,

3. *O. lepidus* was able to discriminate differentially against self and conspecific parasitised hosts, whereas *A. subandinus* discriminated equally both types of parasitised hosts,

4. *O. lepidus* was a better competitor than *A. subandinus* in multiparasitised hosts,

5. *O. lepidus* had the advantage of a longer ovipositor than *A. subandinus* and was therefore able to access hosts in long mines, and left a lower percentage of unparasitised hosts when searching under equivalent conditions,

6. *A. subandinus* developed faster than *O. lepidus*,

7. both species develop faster and have greater fecundity than their host,

8. both species are well-adapted to their local environment over a range of temperatures from 15°C to 35°C, but *A. subandinus* was more tolerant of low and high temperatures than *O. lepidus*.

These results suggest that in an augmentative biological control program of PTM, it is an advantage to release both species, firstly *A. subandinus* followed by *O. lepidus*. One may choose the single species introduction of *O. lepidus*, because it seems to be more effective at maintaining PTM populations at a low level. However, the results indicated that the two parasitoids play a complementary role in the control of PTM and this is related to their responses to both biotic and abiotic factors.

10.2 Conclusions

The outcome of this study supports the hypothesis that both biotic and abiotic factors influence the efficiency of the two parasitoids. For a successful augmentative biological control program of PTM, it is necessary to consider how a variety of factors influence the efficacy of these parasitoids and to plan releases to increase the rate of parasitism in the field.

Within suitable habitats, the highest rate of parasitism by the two species occurred on newly established first instar larvae, otherwise at least 9% of hosts escape parasitism in wider mines in the plant tissue (Section 9.3). Thus, prediction of release time is essential and could be synchronised to the onset of PTM egg hatch. This peak may be estimated using pheromone traps (Section 1.3.4), day degree predictions using the developmental threshold temperature of PTM (Appendix 1.3), or historical records using information on the biology of PTM in different areas.

The number of wasp releases could be estimated using population data of PTM, and relating this to moth oviposition (with an average of 104 eggs during a female's lifetime). Information from the studies on the reproductive efficiency of the two species (Chapter 4), could possibly be used to estimate the number of parasitoids required to successfully control the populations of PTM in each area. However, synchrony between the population of the first instar larvae of PTM and adult parasitoids is an essential factor. Previous experiences in the field (P.A. Horne, pers. comm.) indicate that releases of pupae of these parasitoids are advantageous. Thus, information on the developmental period of the pupal stage (Section 3.3) aids in estimating the release time to correspond with the onset of egg hatching of PTM.

Although the dispersal ability of the two parasitoids was not studied in the field, information from the studies on searching efficiency and host finding (Chapters 6 and 7) help to estimate the most effective distance between release cages. Since there is synchrony between the generations of these parasitoids and PTM, the number of releases is dependent on the number of PTM generations in each area. Farmers in many countries, including Australia, follow an extensive pattern of crop rotation, and migration of parasitoids across long distances from old plantings to new may be virtually impossible. Thus, an annual release of parasitoids may be essential for *reliable* biological control in infested crops.

The results of host discrimination by the two species indicated that both species incur superparasitism (Chapter 9), depending on host location, the period between first and second parasitism, duration of exposure and host density. *O. lepidus* is one of only a few species that is known to have the ability of self host discrimination. These results suggested an initial release schedule should release *A. subandinus* in cold areas or at the beginning of the growing season, and, where release of both species is contemplated, *A. subandinus* could be released before *O. lepidus*. *A. subandinus* seems to be more able to maintain its population when the host population is at a low level (Chapter 6), and may decrease PTM populations at the

beginning of the growing season when *O. lepidus* is less active. Later in the season with increasing temperatures, *A. subandinus* and *O. lepidus* play complementary roles in keeping the host population at a low level.

10.3 Suggestions

Clearly, the influence of the factors that affect the efficiency of the two parasitoids needs further investigation in the field. The information obtained from this study will serve as a guide for further studies as summarised below.

1) There are no publications about the interactions between *A. subandinus* and *O. lepidus* and other parasitoids, particularly the egg parasitoid *Copidosoma* spp. In particular an investigation of whether *A. subandinus* and *O. lepidus* accept or reject hosts parasitised by *Copidosoma* is warranted.

2) Although the two parasitoids show multiparasitism, the results support multiple or successive introduction and are in agreement with many practical and theoretical biological workers (Smith, 1929; Huffaker and Kennet, 1966; DeBach and Huffaker, 1971; Hassell, 1978; May and Hassell, 1981). The results from self-, con- and inter-specific host discrimination (Chapter 9) could be supported by means of a good mathematical model to clarify the role of host discrimination in the ability of these parasitoids to be effective biological control agents. However, the models presented by Hassell and Varley (1969) and Munster-Swendsen, (1982) cover only a special case with respect to niche overlaps between parasitoids.

3) A study to determine the events and mechanisms that may influence the effectiveness of a mass release program of the two species in the field, especially their persistence when initial host densities are high or low.

4) Although the research undertaken on host discrimination by the two parasitoids in this thesis succeeded in identifying numerous behavioural mechanisms, and clarified some important issues involved in self-, con- and inter-specific host discrimination, the exact mechanisms of discrimination were not identified. Such mechanisms need to be further explored.

5) Classical control using the parasitoids of PTM has been shown to give good results in potato crops in many areas (Horne, 1990b; Siddig, 1990; University of California, 1992; Fuglie *et al.*, 1993). However, the wasps will give better results where they are part of an IPM strategy. Such an IPM strategy has been developed in Australia by Horne and co-workers (Horne, 1993) and relies heavily on parasitoids, predators and cultural control measures. The cultural controls include good overhead irrigation, pathogen tested seed, and control of solanaceous weeds and volunteer potatoes. It has been suggested by Horne (1993) that inundative releases of wasps could be a valuable addition to this existing IPM strategy. Commercial releases of insectary-reared *O. lepidus* are now being made in potato crops in Queensland, NSW, Victoria and Western Australia (P.A. Horne, pers. comm.). This approach could also have applications in other solanaceous crops such as tomatoes and tobacco, but needs further research.

It is hoped that the suggestions and questions raised during the course of this project will be considered for future study.

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Appendix

Appendix 1.1: Insect pests of potato recorded in Australia (Hely *et al.*, 1982; Cantrell *et al.*, 1983 and Raman and Radcliffe, 1992).

Scientific name	Order	Common name
Phaulacridium vittatum Sjostedt	Orthoptera	Wingless grasshopper
Gryllotalpa spp.	Orthoptera	Mole crickets
Teleogryllus commodus Walker	Orthoptera	Black field cricket
Teleogryllus oceanicus Le Guillou	Orthoptera	Field cricket
Aphis gossypii Glover	Homoptera	Cotton aphid
Myzus persicae Sulzer	Homoptera	Green peach aphid
Macrosiphum euphorbiae Thomas	Homoptera	Potato aphid
Austroasca viridigrisea Paoli	Homoptera	Vegetable leafhopper
Austroagallia torrida Evans	Homoptera	Spotted leafhopper
Austroasca alfalfae Evans	Homoptera	Lucerne leafhopper
Bemisia tabaci Gennadius	Homoptera	Tobacco whitefly
Nezara viridula L.	Heteroptera	Green vegetable bug
Nysius vinitor Bergroth	Heteroptera	Rutherglen bug
Creontiades dilutus Stal.	Heteroptera	Green mirid
Frankliniella occidentalis	Thysanoptera	Flower thrips
Frankliniella schultzei Trybom	Thysanoptera	Tomato thrips
Thrips tabaci Lindemann	Thysanoptera	Onion thrips
Graphognathus leucoloma Boheman	Coleoptera	White fringed weevil
Listroderes obliquus Klug	Coleoptera	Vegetable weevil
Henosepilachna sparsa-vigintisexpunctata Boisduval	Coleoptera	Twenty-six-spotted potato ladybird
Heteronychus arator Fabricius	Coleoptera	African black beetle
Elateridae	Coleoptera	Wireworms
Gonocephalum carpentariae Blackburn	Coleoptera	False wireworms
Gonocephalum macleayi Blackburn	Coleoptera	False wireworms
Halticinae	Coleoptera	Flea beetles
Rhopaea magnicornis Blackburn	Coleoptera	Large pasture scarab
Gonocephalum carpentariae Blackburn	Coleoptera	Northern false wireworm
Gonocephalum macleayi Blackburn	Coleoptera	Southern false wireworm
Agrotis spp.	Lepidoptera	Cutworms
Spodoptera litura F.	Lepidoptera	Cluster caterpillar
Chrysodeixis spp.	Lepidoptera	Loopers
Phthorimaea operculella Zeller	Lepidoptera	Potato tuberworm

Appendix 1.2: Summary of the morphology of PTM and two of its parasitoids (Rothschild, 1986; Povolny, 1991; Oatman *et al.*,1969; Cardona and Oatman, 1975).

Stage	*Apanteles subandinus* *		*Orgilus lepidus* **		*Phthorimaea operculella* ***	
	colour	size (mm)	colour	size (mm)	colour	size (mm)
Egg	white	0.32	white	0.26	white	0.5
1st instar	white	1.33	white	3.01	black	1.0
2nd instar	creamy-white	2.27	creamy-white	4.10		
3rd instar	creamy-white	3.24	creamy	5.6		
4th instar					greenish-pink	12.0
Prepupa	creamy-white	3.8	creamy			
Pupa ***	creamy-white with spots	3.6	creamy	5.1	pale brown	8.0
Adult	black	3.6-3.8	yellowish	4-4.5	greyish-brown	12-16
Ovipositor	black	0.63	black	3.5		

* Cardona and Oatman (1975)
** Oatman *et al.* (1969)
*** Rothschild (1986)

Appendix 1.3 Optimal temperature range and humidity for PTM and two of its parasitoids (Oatman *et al.*,1969; Cardona and Oatman, 1975; Briese, 1986; Markosyan, 1993).

Insect	Minimum Temperature °C (Threshold)	Optimal Temperature (°C)	Maximum Temperature (°C)	Optimal % RH.
PTM	4.25-9.5	24-29	35-38	50
O. lepidus	_	26	_	50
A. subandinus	15	29.5	32	50

Appendix 1.4: Host plants of potato moth (based on published records, e.g. Rothschild, 1986).

Scientific name	**Common name**
Solanum tuberosum L.	potato
S. melongena L.	egg plant
Lycopersicon esculentum Miller	tomato
Nicotiana tabacum L.	tobacco
Capsicum spp.	pepper
Physalis peruviana L.	cape gooseberry
Physali minima L.	wild gooseberry
Nicandra physalodes L.	false cape gooseberry
Datura stramonium L.	common thornapple
Datura metel L.	hoary or Hindu thornapple
Nicotiana glauca Grah	tree tobacco
Nicotiana debneyi Domin.	-
Nicotiana goodspeedii Wheeler	small-flowered tobacco
Nicotiana. megalosiphon Hue.& Muell	-
Solanum aculeatissicum Jacq.	-
Solanum cinereum R. Br.	Narrawa burr
Solanum nigrum L.	blackberry nightshade
Solanum hermannii Dunal	apple of sodom
Solanum torvum Sw.	devil's fig
Solanum verbascifolium L.	

Appendix 1.5: Parasitoids of PTM.

Scientific name	Familly	Source
Hymenoptera		
**Agathis gibbosa* Say	Aconidae	Oatman & Platner, 1974; Lloyd, 1972
Agathis tandilensis Blanchard	Aconidae	Oatman & Platner, 1989
Agathis unicolor Schrottky	Aconidae	Oatman & Platner, 1989
Agathis unicolorata Shenefelt	Aconidae	Izhevskiy, 1986; Oatman &Platner, 1989
Apanteles appellatorate	Braconidae	Oatman & Platner, 1974
Apanteles carpatus Say	Braconidae	Izhevskiy, 1986
Apanteles dignus Muesebeck	Braconidae	Izhevskiy, 1986; Oatman&Platner, 1989
Apanteles gelechiidivorus Marsh.	Braconidae	Izhevskiy, 1986
Apanteles litae Nixon	Braconidae	Salama *et al.*, 1996
** *Apanteles melanoscelus* Ratzeburg	Braconidae	Rothschild, 1986
Apanteles scutellaris Muesebeck	Braconidae	Lloyd, 1972; Oatman & Platner, 1974
**Apanteles subandinus* Blanchard	Braconidae	Izhevskiy, 1986; Oatman &Platner, 1989
Apsilophrys oeceticola DeSantis	Braconidae	Oatman & Platner, 1989
Bracon cuyanus Blanchard	Braconidae	Oatman & Platner, 1989
Bracon brevicornis Wesm.	Braconidae	Izhevskiy, 1986
Bracon hebetor Say	Braconidae	Izhevskiy, 1986
**Bracon gelechiae* Ashmead (*Habrobracon johannseni* Viereck)	Braconidae	Oatman & Platner, 1974; Sankaran & Girling, 1980; Lloyd, 1972
Bracon instabilis Marsh.	Braconidae	Salama *et al.*, 1996
Cardiochiles explorator Say	Braconidae	Izhevskiy, 1986
Chelonus blackburni Cameron (*Microchelonus phthorimaea* Gahan)	Braconidae	Platner & Oatman, 1972; Ballal&Kumar, 1991; Oatman & Platner, 1989; Lloyd, 1972
Chelonus caucasicus	Braconidae	Oatman & Platner, 1989
Chelonus curvimaculatus Cameron	Braconidae	Mitchell, 1978; Oatman & Platner, 1989; Whiteside, 1980
Chelonus derpessus Thomson	Braconidae	Izhevskiy, 1986
Chelonus kellieae Marsh	Braconidae	Oatman & Platner, 1989; Sankaran & Girling, 1980
Chelonus shoshoneanorum Viereck	Braconidae	Oatman & Platner, 1974
Chelonus subcontractus	Braconidae	Oatman & Platner, 1989
Hormius pallidipes Ashm.	Braconidae	Izhevskiy, 1986
Macrocentrus ancylivorus Rohwer	Braconidae	Lloyd, 1972; Izhevskiy, 1986
Meteorus vulgaris Cress.	Braconidae	Izhevskiy, 1986
***Microgaster curvimaculatus* Cameron (*Microchelonus*)	Braconidae	Sankaran & Girling, 1980
Microchelonus contractus Nees	Braconidae	Izhevskiy, 1986
Microgaster phthorimaea Muesebeck	Braconidae	Oatman &Platner, 1974; Izhevskiy, 1986
Microplitis minuralis Muesebeck	Braconidae	Izhevskiy, 1986; Oatman &Platner, 1989
Orgilus jennieae Marsh	Braconidae	Oatman & Platner, 1989; Sankaran & Girling, 1980
Orgilus californicus Provancher	Braconidae	Oatman & Platner, 1989

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Appendix 1.5: (Continued from previous page).

Scientific name	Familly	Source
Hymenoptera		
Orgilus lateralis Cresson	Braconidae	Oatman & Platner, 1989
**Orgilus lepidus* Muesebeck	Braconidae	Oatman & Platner, 1974; Izhevskiy, 1986
Orgilus mellipes Say	Braconidae	Izhevskiy, 1986
Orgilus parcus Turner	Braconidae	Oatman & Platner, 1989; Whiteside, 1980
Orgilus pimpinellae	Braconidae	Oatman & Platner, 1989
Parahormius pllidipes	Braconidae	Oatman & Platner, 1974, Oatman & Platner, 1989
Rogas spp.	Braconidae	Oatman & Platner, 1989
** *Pristomerus spinator* Fabricius	Chalcididae	Oatman & Platner, 1974
Pristomerus vulnerator	Chalcididae	Lloyd, 1972
***Proconura sp.*	Chalcididae	Oatman & Platner, 1974
***Elasmus funereus* Riek	Elasmidae	Oatman & Platner, 1974
Copidosoma desantisi Annecke & Mynhardt	Encyrtidae	Sankaran & Girling, 1980
**Copidosoma koehleri* Blanchrd	Encyrtidae	Oatman & Platner, 1974; Lloyd, 1972
Copidosoma oeceticola D. S.	Encyrtidae	Izhevskiy, 1986
Copidosoma phthorimaeae Cushman	Encyrtidae	Lloyd, 1972; Izhevskiy, 1986
Copidosoma urunguayensis Tachikawa	Encyrtidae	Mitchell, 1978
Elachertus sp.	Eulophidae	Oatman & Platner, 1989
Elasmus funereus Riek.	Eulophidae	Izhevskiy, 1986
Hyssopus sp	Eulophidae	Oatman & Platner, 1989
Rhetisympiesis	Eulophidae	Oatman & Platner, 1989
*phthorimaeae*Blanchard	Eulophidae	Oatman &Platner, 1974; Izhevskiy, 1986
* *Sympiesis stigmatipennis* Girault		
Zagrammosoma flavolineatum Crawford	Eulophidae	Oatman & Platner, 1974; Izhevskiy, 1986
Campoletis flavicincta Ashmead	Ichneumonidae	Izhevskiy, 1986
Campoplex haywardi Blanchard	Ichneumonidae	Oatman&Platner, 1974; Lloyd,
**Campoplex phthorimaea* Cushman	Ichneumonidae	1972; Oatman & Platner, 1974;
Diadegma blackburni Cameron	Ichneumonidae	Izhevskiy,1986
Diadegma compressus Cresson	Ichneumonidae	Izhevskiy, 1986; Oatman & Platner, 1989
Diadegma fenestralis Holmgr.	Ichneumonidae	Izhevskiy, 1986
Diadegma insulare Cress	Ichneumonidae	Izhevskiy, 1986

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Appendix 1.5: (Continued from previous page).

Scientific name	Familly	Source
Hymenoptera		
Diadegma mulipum Holmgren (*Diadegma stellenboschense* Cameron)	Ichneumonidae	Whiteside, 1980; Sankaran & Girling, 1980
Diadegma raoi Gupta	Ichneumonidae	Oatman & Platner, 1974; Sankaran & Girling, 1980
Diadegma surendrai Gupta	Ichneumonidae	Izhevskiy, 1986
Diadegma turcato Aubert	Ichneumonidae	Izhevskiy, 1986
Eriborus trochanteratus Morl.	Ichneumonidae	Sankaran & Girling, 1980
Itoplectis conquisitor Say	Ichneumonidae	Sankaran & Girling, 1980
	Ichneumonidae	Izhevskiy, 1986
Nepeira fuscifemora Graf	Ichneumonidae	Oatman & Platner, 1989
Nythobio stellenboschenis Cameron	Ichneumonidae	Izhevskiy, 1986
Pimpla aequalis Prov.	Ichneumonidae	Mitchell, 1978
Pristomerus spinator Fabricius	Ichneumonidae	Izhevskiy, 1986
Pristomerus vulnerator Panz.	Ichneumonidae	Izhevskiy, 1986
Scambus hispae Harris	Ichneumonidae	Izhevskiy, 1986
**Temelucha forester*	Ichneumonidae	Izhevskiy, 1986
***Temeluch aminuta* Morley	Ichneumonidae	Oatman & Platner, 1989
Temelucha picta Girault	Ichneumonidae	Whiteside, 1980
Temelucha platensis	Ichneumonidae	Oatman & Platner, 1989
Anaphes sp.	Mymaridae	Izhevskiy, 1986
Perilampus fulvicornis Ashmead	Perilampidae	Izhevskiy, 1986
Perilampus granulosus Crwf.	Perilampidae	Izhevskiy, 1986
Dibrachys cavus Walker	Pteromalidae	Izhevskiy, 1986
Dibrachys clisiocampae Fitch	Pteromalidae	Izhevskiy, 1986
Dibrachys turcattor Aubert	Pteromalidae	Izhevskiy, 1986
Habrocytus cerealella Ashmead	Pteromalidae	Izhevskiy, 1986
Telenomus heliotidis	Scelionidae	Izhevskiy, 1986
Trichogramma sp.	Trichogrammatidae	Izhevskiy, 1986
Diptera		
Tachina sp.	Tachinidae	Oatman & Platner, 1974 & 1989
Lixophaga diatraecae Towns	Tachinidae	Izhevskiy, 1986
Incamyia cuzcensis T.T.	Tachinidae	Lloyd, 1972
Nematoda		
Hexamermis sp.	Mermithidae	Oatman & Platner, 1989

* Exotic wasp in Australia.
** Indigenus wasp in Australia.

Appendix 1.6: Predators of PTM recorded from Australia (based on published records, e.g. Cantrell *et al.*, 1983; Kroschel and Koch, 1994).

Scientific name	Familly	Order
Adonia variegata Goeze	Coccinellidae	Coleoptera
Allograpta spp.	Syrphidae	Diptera
Bembidion spp.	Carabidae	Coleoptera
Betasyrphus spp.	Syrphidae	Diptera
Broscus spp.	Carabidae	Coleoptera
Cheilomenes lunata F.	Coccinellidae	Coleoptera
Cheilomenes propinaqua vicina Mulsant	Coccinellidae	Coleoptera
Chrysopa carnea	Chrysopidae	Neuroptera
Chrysopa rufilabris	Chrysopidae	Neuroptera
Chrysopa zastrowi	Chrysopidae	Neuroptera
Chrysotus spp.	Dolichopodidae	Diptera
Coccinella undecimpunctata aegyptica Reiche	Coccinellidae	Coleoptera
Geocoris punctipes	Lygaeidae	Heteroptera
Geocoris tricolor	Lygaeidae	Heteroptera
Ischiodon aegyptius Wied	Syrphidae	Diptera
Lioadalia flavomaculata	Coccinelidae	Coleoptera
Melanostoma scalare Fabr.	Syrphidae	Diptera
Metasyrphus corollae Fabr.	Syrphidae	Diptera
Pirates strepitans RB.	Reduviidae	Heteroptera
Psilopus spp.	Dolichopodidae	Diptera
Psyllobora bisoctonotata Mulsant	Coccinellidae	Coleoptera
Pterostichus spp.	Carabidae	Coleoptera
Pullus mediterraneus Khnzorian	Coccinellidae	Coleoptera

Appendix 1.7: Pathogens of PTM (based on published records, e.g. Thomas and Poinar, 1973).

Scientific name	Stage of PTM
Acerobacter sp.	larva
Bacillus cereus	larva
Bacillus thuringiensis var *thuringiensis*	larva
Granulosis Virus	larva
Neoaplectana carpocapsae	larva
Nosema sp.	larva
Serratia marcescens Bizio	larva
Streptococcus sp.	larva
Streptococcus faecalis	larva

Appendix 2.1: Average wind speed in the Open Wind Tunnel arena with dial set on number 2.

Position	The wind speed (cm/s) at height indicated							
	10 cm		**20 cm**		**30 cm**		**Overall**	
Column	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD
I								
Column1	19	4.5	12	5.5	28	1.0	20	3.7
Column2	24	5.1	31	2.5	18	0.6	24	2.7
Column3	23	2.1	27	4.6	21	2.0	24	2.9
II								
Column1	12	1.2	16	4.7	19	2.0	16	2.6
Column2	27	6.0	34	2.6	26	6.6	29	5.1
Column3	27	1.0	28	3.6	29	1.2	28	1.9
III								
Column1	14	5.0	14	3.0	14	3.2	14	3.7
Column2	25	5.6	31	1.5	26	6.8	27	4.6
Column3	20	1.2	26	3.5	28	1.5	25	2.1
IV								
Column1	18	5.7	12	1.0	13	1.5	14	2.7
Column2	27	9.0	24	11.4	22	8.5	24	6.3
Column3	22	3.0	28	3.2	26	4.1	25	3.4
V								
Column1	21	6.1	14	0.6	12	1.0	16	2.6
Column2	33	2.9	33	3.7	19	8.9	28	5.2
Column3	24	8.1	31	1.5	27	1.5	27	3.7
Overall								
Column1	16.0	4.5	13.0	3.0	17.0	1.7	15.3	3.0
Column2	27.0	5.7	30.0	4.3	22.0	6.3	26.3	5.4
Column3	23.0	3.1	28.0	3.3	26.0	2.1	25.3	2.8

The average wind speed (i.e. the average of 135 measurements) was 22.4 m/s (stdev =3.7), when fan speed was on dial 2.

Legend : I, II, III, IV, and V indicate the position of the 5 rows slots in the Open Wind Tunnel arena from left to right.

Column 1 Corresponds to the edge nearest to the wall (33cm from the wall)

Column 2 Corresponds to the middle slots line.

Column 2 Corresponds to the edge nearest to the observer (73cm from the wall)

* This is the mean of three measurements.

Appendix 3.1: The mean of development time (days), sd. range and developmental rate of *A. subandinus* at five constant temperatures.

Life stage	Temperature	15°C	20°C	25°C	30°C	35°C
Egg-larva						
	Mean (day)	21.30	17.80	11.80	9.00	7.30
	sd.	2.5	2.1	1.9	1.1	0.9
	Range (day)	17-24	14-20	8-14	7-11	5-11
	Rate (1/day)	0.04	0.05	0.08	0.1	0.14
	n	20	20	20	21	27
Prepupa						
	Mean (day)	2.60	2.40	2.40	1.60	1.45
	sd.	0.17	0.24	0.15	0.28	0.28
	Range (day)	1-3	1-4	1-3	1-3	1-3
	Rate (1/day)	0.42	0.50	0.42	1.00	0.50
	n	20	20	20	21	27
Pupa						
	Mean (day)	13.26	11.60	7.30	5.25	5.70
	sd.	1.00	1.10	0.87	0.63	0.67
	Range (day)	12-15	10-16	6-8	4-6	1-3
	Rate (1/day)	0.08	0.09	0.13	0.20	0.17
	n	19	20	20	20	23
Mean egg-adult		**36.93**	**31.8**	**21.50**	**15.85**	**14.68**

Appendix 3.2: The mean of development time (days), sd. range and developmental rate of *O. lepidus* at five constant temperatures.

Life stage	Temperature	15°C	20°C	25°C	30°C	35°C
Egg-larva						
	Mean (day)	21.60	18.30	14.13	9.75	8.75
	sd.	2.50	1.80	3.40	2.00	1.80
	Range (day)	14-25	16-21	9-22	7-15	5-13
	Rate (1/day)	0.05	0.06	0.08	0.11	0.13
	n	27	28	30	40	41
Prepupa						
	Mean (day)	3.40	2.80	2.60	1.80	2.00
	sd.	1.1	0.6	1.1	0.9	1.0
	Range (day)	2-5	2-4	1-4	1-4	1-5
	Rate (1/day)	0.25	0.33	0.33	0.50	0.50
	n	27	28	30	36	27
Pupa						
	Mean (day)	12.75	11.10	9.60	6.70	7.80
	sd.	2.5	3.3	1.7	1.0	3.3
	Range (day)	7-17	6-16	7-14	5-8	5-13
	Rate (1/day)	0.08	0.09	0.11	0.14	0.17
	n	27	28	30	36	12
Mean egg-adult		**37.75**	**32.2**	**26.33**	**18.25**	**18.55**

Appendix 3.3: The mean of development time (days), sd., range and developmental rate of PTM at five constant temperatures.

Life stage	Temperature	15°C	20°C	25°C	30°C	35°C
Egg						
	Mean (day)	10.3	7.1	4.3	3.0	2.5
	sd.	0.01	0.01	0.02	0.00	0.08
	Range (day)	10-11	7-7.5	4-4.5	3-3	2-3
	Rate (1/day)	0.10	0.14	0.22	0.33	0.40
	n	20-25	20-25	20-25	20-25	20-25
Larvae						
	Mean (day)	24.0	21.2	14.0	10.1	9.4
	sd.	2.12	1.72	1.07	1.44	1.14
	Range (day)	18-26	17-24	13-16	7-12	7-12
	Rate (1/day)	0.04	0.05	0.07	0.10	0.11
	n	22	22	28	24	34
Prepupa						
	Mean (day)	3.5	3.0	2.6	2.0	1.3
	sd.	1.49	1.29	0.80	1.08	0.60
	Range (day)	2-6	2.5	1-4	1-4	1-3
	Rate (1/day)	0.28	0.33	0.50	0.50	1.00
	n	22	22	26	23	23
Pupa						
	Mean (day)	15.1	10.1	7.9	5.6	4.4
	sd.	1.98	1.39	0.76	0.91	0.62
	Range (day)	8-18	9-13	7-9	4-7	3-5
	Rate (1/day)	0.06	0.10	0.13	0.17	0.25
	n	22	20	25	21	17
Mean larvae-adult		**42.6**	**34.3**	**24.5**	**17.7**	**15.1**
Mean Egg-adult		**52.9**	**41.4**	**28.8**	**20.7**	**17.6**

Appendix 4.1: The number of eggs in *O. lepidus* ovaries at different age of females.

Range of age	female no.	no. of ovarioles	Total eggs	Mean (± SE)
4-12-hr	1	9	144	**117 (±14.6)**
	2	8	94	
	3	8	112	
1-day-old	1	12	176	**129 (±21.0)**
	2	6	96	
	3	8	148	
	4	8	160	
	5	6	64	
2-6-day-old	1	9	144	**144 (±13.2)**
	2	8	176	
	3	8	154	
	4	8	96	
	5	8	116	
	6	12	176	
7-10-old	1	8	160	**158 (±1.2)**
	2	8	156	
	3	8	160	
	4	8	156	
11-13-old	1	8	128	**156 (±16.6)**
	2	6	96	
	3	8	112	
	4	12	208	
	5	8	126	
	6	8	224	
	7	8	176	
	8	8	176	
14-18-day-old	1	8	186	**129 (±12.5)**
	2	12	208	
	3	8	132	
	4	8	116	
	5	12	192	
	6	8	96	
	7	8	112	
	8	8	160	
	9	6	90	
	10	8	156	
	11	8	64	
	12	8	176	
	13	8	140	
	14	6	40	
	15	8	128	
	16	8	60	

Appendix 4.2: The number of eggs in *A. subandinus* ovaries at different age of females.

Range of age	female no.	Total eggs	Mean (± SE)
4-12-hr	1	112	**79 (± 9.0)**
	2	108	
1-day-old	1	134	**121 (± 13.0)**
	2	108	
4-8-day-old	1	114	**123.6 (± 13.9)**
	2	152	
	3	130	
	4	70	
	5	152	
10-13-day-old	1	88	**110.8 (± 16.5)**
	2	64	
	3	148	
	4	158	
	5	96	
14-16-old	1	92	**100.4 (± 18.9)**
	2	28	
	3	152	
	4	124	
	5	106	
17-old	1	102	**110.7 (± 8.7)**
	2	128	
	3	102	