STUDIES ON PARASITOIDS OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE), IN SOUTH AFRICA

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By

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Table of contents

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Abstract

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major pest of *Brassica* crops in many parts of the world. Because of its ability to develop resistance to virtually all major groups of insecticides, including *Bacillus thuringiensis* Berliner (Bt), much attention has therefore been given to biological control using parasitoids. South Africa has an abundance of parasitoids attacking this pest. *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) is the most abundant larval parasitoid of *P. xylostella* in South Africa. In East Africa, its role in biological control of *P. xylostella* is insignificant, and the most abundant parasitoid there is *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), a larval-pupal parasitoid. In South Africa, however, *D. mollipla* is out-competed by *C. plutellae*. Total parasitism of *P. xylostella* in East Africa rarely exceeds 15%, therefore there is a need to introduce more effective and heat-tolerant parasitoids of *P. xylostella* to that region. This study was therefore initiated to examine the potential of *C. plutellae* and *D. mollipla* as biological control agents by studying certain aspects of their biology in the laboratory, as well as the suitability of *C. plutellae* for introduction into East Africa. Biological aspects studied were: (i) host instar preference, fecundity, and searching efficiency of *C. plutellae* and *D. mollipla* at different host and parasitoid densities; (ii) effects of temperature on parasitism of *P. xylostella* by *C. plutellae* and *D. mollipla*, and on their rates of development and emergence. In addition, the role of parasitoids in controlling *P. xylostella* on unsprayed cabbage plots was investigated.

Both *C. plutellae* and *D. mollipla* preferred to attack second and third instar hosts than fourth instars in *choice* and *no-choice* tests. However, *D. mollipla* attacked more fourth instar hosts than *C. plutellae*. *Cotesia plutellae* laid mainly female eggs in second and third instar hosts than in fourth instars, whereas *D. mollipla* laid more female eggs in fourth instar hosts than in second and third instar hosts. *Diadegma mollipla* was more fecund [82.57 ± 32.87, (mean ± s.d.) than *C. plutellae* (42.13 ± 12.2), and was long lived (7.13 ± 3.69 days) compared to the latter (5.23 ± 2.7 days). An increase in host density resulted in the reduction in the area of discovery (*a*) and the killing power (*K*) for both parasitoids. No significant differences were detected between the searching efficiency ($t = -1.42$NS, d.f. = 48, $P < 0.001$) of the two parasitoids. An increase in parasitoid density also resulted in a decline in searching efficiency, but not the killing power, of both parasitoids. *Cotesia plutellae* completed development at all temperatures tested (21-33°C), whereas *D. mollipla* completed development at temperatures

from 18-30°C, and *C. plutellae* had a lower threshold for development (8.14°C) compared to *D. mollipla* (10.23°C). At all tested temperatures, the generation time for *C. plutellae* was shorter compared to *D. mollipla*. The possible reasons for the dominance of *C. plutellae* over *D. mollipla* in the field are: shorter generation time, high production of female progeny in younger hosts, low interference among searching females, and relatively wide thermal tolerance.

The role of parasitoids in regulating diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), populations was studied for two years (February 2000-January 2002) on unsprayed cabbage fields at Brits, North West Province, South Africa. Cabbage seedlings were transplanted in three consecutive times each year. Cabbage infestations by *P. xylostella* larvae and pupae, and their parasitoids, were monitored at weekly intervals. The flight activity of *P. xylostella* male moths was monitored using sex-pheromone traps. Trap catches indicated that the moths were active throughout the year. The flight activity of the moths corresponded with infestations on the crop. Trap catches and infestation levels were generally low from December to August and high from September to November. Eight hymenopteran parasitoids were reared: the larval parasitoids *Cotesia plutellae* (Kurdjumov) (Braconidae) and *Apanteles halfordi* (Ullyett) (Braconidae); the larval-pupal parasitoids *Oomyzus sokolowskii* (Kurdjumov) (Eulophidae) and *Diadegma mollipla* (Holmgren) (Ichneumonidae); the pupal parasitoid *Diadromus collaris* (Gravenhorst) (Ichneumonidae); and the hyperparasitoids *Eurytoma* sp. (Eurytomidae), *Mesochorus* sp. (Ichneumonidae), and *Pteromalus* sp. (Pteromalidae). *Cotesia plutellae* was the most abundant parasitoid of *P. xylostella* followed by *O. sokolowskii*, *D. collaris*, *A. halfordi* and *D. mollipla.* Parasitism of *P. xylostella* larvae was high reaching 100% on several occasions during late spring to end of autumn (November-May) each year. Parasitism was lower (<50%) in winter and early spring (June-September). *Apanteles halfordi* was absent in winter but re-appeared in spring. Parasitism of *P. xylostella* pupae by *D. collaris* was high only during spring (September-October). Hyperparasitism was generally low except when primary parasitoids were abundant in spring (September-November) and summer (December-February) when up to 25% of *P. xylostella* larvae and *C. plutellae* cocoons yielded hyperparasitoids. The role of other biotic and abiotic mortality factors on the population dynamics of *P. xylostella* is discussed.

Declaration

I declare that this work is an original version of my studies and has never been submitted anywhere else.

Chapter 1:

General introduction

1.1 Background

The plant family Brassicaceae is a large, diverse and widespread plant group that includes economically important crops such as cabbage, kale, cauliflower, canola, broccoli, mustard, and Chinese cabbage that are widely grown throughout the world (Talekar & Shelton 1993). One of the serious constraints to the successful production of these crops are insect pests; some of which are important only in specific climates while others occasionally inflict serious damage (Annecke & Moran 1982; Lim *et al.* 1997). The diamondback moth, *Plutella xylostella* (L.), is regarded as the most destructive insect pest of *Brassica* crops throughout the world (Anonymous 1987; Waterhouse & Norris 1987; Talekar & Shelton 1993). The larvae are voracious defoliators with a potential to destroy the entire crop if left uncontrolled (Kibata 1997). Since the introduction of DDT in the 1940s, efforts to control *P. xylostella* have relied heavily on insecticides (Talekar & Shelton 1993). However, high insecticide inputs have often resulted in the development of resistance. In an attempt to overcome resistance, farmers increase doses and/or use mixtures of several insecticides, spraying repetitively every second or third day (Ooi 1980; Talekar & Yang 1991; Sereda *et al*. 1997). This activity has led *P. xylostella* to develop multiple- and cross-resistance to a wide range of insecticides (Ooi 1980; Talekar & Yang 1991) and to date resistance to virtually all major groups of insecticides has been documented (Perez *et al.* 1995). *Plutella xylostella* was also the first insect pest to develop resistance to formulations of the microbial insecticide *Bacillus thuringiensis* Berliner in the field (Tabashnik *et al*. 1990; Iqbal *et al.* 1996). Presently, insecticide-resistant populations of *P. xylostella* are causing serious economic losses in many countries, especially those with tropical climates such as Southeast Asia, Pacific Islands, Central America and the Caribbean Islands (Alam 1992; Talekar & Shelton 1993). This serious pest status of *P. xylostella* in the tropics is mainly due to favourable conditions for development and the year-round cultivation of brassicas (Talekar & Shelton 1993). Insecticide resistance is a serious problem not only in the tropics but also in temperate climates (Talekar & Yang 1993), including sub-Saharan Africa (Kibata 1997; Sereda *et al.* 1997).

The management of insecticide resistance in *P. xylostella* has been of major interest in recent years (Magaro & Endelson 1990; Tabashnik *et al.* 1994; Regupathy 1997; Roush 1997). Some of these studies indicate that, although *P. xylostella* develops resistance to insecticides quickly, resistance can disappear when the insect is removed from the selection pressure of the insecticide. However, as is to be expected, the reverted colonies respond rapidly to reselection for resistance (Tabashnik *et al.* 1994). One way to manage or delay the

development of resistance in *P. xylostella* is to use insecticides only when needed, but this requires the setting up of action thresholds (Maltais *et al*. 1998). Therefore, an alternative method of control is needed that is able to act against *P. xylostella* in a density-dependent manner that does not select for resistance.

Biological control, particularly the use of parasitoids, is seen as one of the most important means of reaching this goal. This is especially true where control with insecticides has failed (Talekar & Shelton 1993). Investigations of the pest status of *P. xylostella* in different parts of the world indicate that, despite its wide climatic adaptability, it appears to be held in check by parasitoids in some countries (Lim 1986). Countries that continue to be plagued by *P. xylostella* appear to share the same problem- the absence or ineffectiveness of indigenous parasitoids (Lim 1986). The importance of parasitoids in the management of *P. xylostella* has been witnessed by the reduction of damage after one or more parasitoid species have been introduced into an area where native parasitoids were either ineffective or absent (Poelking 1986; Sastrosiswojo & Sastrodihardjo 1986; Talekar *et al*. 1986; Ooi 1992; Kfir & Thomas 2001). Parasitoids are, in general, host-specific and can parasitise several hundred hosts in a density-dependent manner (Annecke & Moran 1982). In this way, the frequency of insecticidal applications can be greatly reduced (Ooi 1980; Talekar & Yang 1991). Furthermore, the importance of parasitoids is based on the premise that they not only constitute the main factor that is capable of regulating insect pests, but are also a natural resource that is usually self-perpetuating (Lim 1986).

1.2 Motivation for the study

Because *P. xylostella* is oligophagous- feeding only on crops in the plant family Brassicaceae (Talekar & Shelton 1993), and because most cultivated brassicas originate from Europe, it was assumed that *P. xylostella* may have originated from the same area (Hardy, 1938 in Kfir 1998). Therefore, explorations for *P. xylostella* parasitoids focused mainly in Europe (Waage & Cherry 1992; Kfir 1998). The most important parasitoids of *P. xylostella* in Europe, namely *Diadegma semiclausum* (Hellen) (= *D. eucerophaga* Horstmann) (Hymenoptera: Ichneumonidae), *Diadromus* (= *Thyraeella*) *collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) and *Cotesia* (= *Apanteles*) *plutellae* (Kurdjumov) (Hymenoptera: Braconidae), were introduced into several countries around the world where they, in most instances, reduced the damage caused by *P. xylostella* (Lim 1986; Waterhouse & Norris 1987; Talekar & Shelton 1993). However, the introduction of these parasitoids into Southeast Asia,

the major brassica-producing region in the world, showed the importance of climatic matching when introducing parasitoids, an issue that has been overlooked in the past. The tropical and subtropical regions of Southeast Asia are divided into two ecological zones, the cool Highland areas and the hot and humid Lowlands (Sastrosiswojo & Sastrodihardjo 1986; Ooi 1992; Talekar 1997; Verkerk & Wright 1997), and it was hoped that the introduced parasitoids would adapt to this wide climatic variation. However, *D. semiclausum* and *D. collaris* became established and are efficient only in the Highlands, which share a somewhat similar climate with Europe (Talekar 1997; Verkerk & Wright 1997) in countries such as Taiwan (Talekar & Yang 1991), Philippines (Poelking 1986), Indonesia (Sastrosiswojo & Sastrodihardjo 1986) and Malaysia (Ooi 1992). Thermal tolerance studies showed that parasitism of *P. xylostella* and survival of *D. semiclausum* (Chua & Ooi 1986) and *D. collaris* (N.S. Talekar, AVRDC, Taiwan, personal communication) are reduced at high temperatures (>25°C), and this explained their failure to exert appreciable control over *P. xylostella* in the Lowlands. The only parasitoid known to tolerate the hot and humid climate of the Lowlands is *C. plutellae* (Kurdjumov) (Ooi 1992; Talekar & Shelton 1993; Verkerk & Wright 1997), which entered the region accidentally probably from Europe (Lim 1982). Unlike the Highlands where the introduced parasitoids managed to curtail *P. xylostella* breeding, in the Lowlands frequent *P. xylostella* outbreaks occur and additional parasitoids are needed to supplement *C. plutellae* (Verkerk & Wright 1997). One possibility is to search for heat-tolerant but more efficient strains of *C. plutellae* (Roush 1997). In addition, the success of a current biological control programme against *P. xylostella* in East Africa also depends on introductions of more efficient and heat-tolerant parasitoids (B. Löhr, ICIPE, Kenya, personal communication).

It has long been known that a parasitoid that is successful against a given pest in one country may fail to provide the same success when introduced into another country. This is largely due to the fact that parasitoids generally have a narrower thermal tolerance than their hosts (Messenger & Bosch 1971; Bosch *et al.* 1992). Messenger & Bosch (1971) give a good account of the importance of biotypes or climatic strains in biological control using the example of a strain of *Trioxys pallidus* (Halliday) (Hymenoptera: Aphidiidae) that was introduced into California from France for the control of the walnut aphid, *Chromaphis junglandicola* (Kalt.). The French strain established only in coastal and intermediate zones of southern California, which share a similar climate with the country of origin. Even after repeated releases, it failed to establish in the Central Valley, leading to a conclusion that the

severe summer and winter climate of this region was the principal limiting factor for this parasitoid, as there were no competitors. It was then decided to import the same parasitoid from Iran. The central plateau of Iran has a similar climate to the Central Valley of California, and within one season the strain from Iran was well established in this area (Messenger & Bosch 1971). From this example it can be deduced that biological control programmes can be greatly enhanced by introductions of parasitoid strains from different ecological zones. Therefore, instead of just concentrating on Europe for *P. xylostella* parasitoids, efforts should also be directed to other countries worldwide, especially where there is evidence that indigenous parasitoids provide good control of *P. xylostella*.

South Africa is predominantly a warm and dry country; these are the climatic conditions that normally lead to *P. xylostella* outbreaks in many countries (Talekar & Shelton 1993). However, the pest status of *P. xylostella* is low in South Africa compared to other countries of similar climate (Kfir 1997). The low pest status is due to the presence of a rich and effective guild of primary parasitoids attacking various developmental stages of *P. xylostella* (Kfir 1997). Knowledge of the importance of indigenous parasitoids in controlling *P. xylostella* in South Africa dates back to the 1930s when Ullyett (1947) conducted the first study in this regard. He reported that *P. xylostella* is a problem only occasionally in South Africa, as it is normally held in check by parasitoids. Parasitic Hymenoptera have a great potential in controlling *P. xylostella* in many parts of the world and the most promising species are *C. plutellae*, *D. semiclausum, D. collaris, Oomyzus sokolowskii* (Kurdjumov) (Eulophidae), *Diadegma insulare* (Cresson) (Ichneumonidae) and *Microplitis plutellae* Muesbeck (Braconidae) (Lim 1986; Talekar & Shelton 1993; Talekar 1997; Xu *et al.* 2001). Except for *D. semiclausum, D. insulare* and *M. plutellae*, all the others are indigenous to, and abundant in, South Africa (Kfir 1997; Waladde *et al.* 2001; Smith 2002; Mosiane *et al.* 2003). The focus of this study, however, is on *C. plutellae* and the African species of *Diadegma*, *D. mollipla* (Holmgren) (Ichneumonidae).

Cotesia plutellae has been recorded in many countries around the world (see section on **introduction to study insects** below). Throughout its range, the biotype from South Africa appears to be the most effective. Firstly, in contrast to other countries where *C. plutellae* is abundant only in low elevations (Talekar & Shelton 1993; Verkerk & Wright 1997), in South Africa it is the most abundant parasitoid both at low (<500m) (Waladde *et al.* 2001) and high elevations (>1000m) (Kfir 1997; Mosiane *et al*. 2003). Secondly, hyperparasitism is often

cited as one of the most important limiting factors to its efficacy in some countries (Alam 1992; Liu *et al.* 2000), but in South Africa *C. plutellae* dominates the parasitoid complex despite hyperparasitism and competition from other primary parasitoids (Kfir 1997; Mosiane *et al.* 2003). Thirdly, the contribution of *C. plutellae* in total parasitism of *P. xylostella* is low in many other countries (Waterhouse & Norris 1987), whereas in South Africa it is the most efficient parasitoid often accounting for more than 80% of total parasitism, which is normally >90% (Kfir 2003). From this evidence, it can be deduced that the South African strain of *C. plutellae* has some traits that are lacking in others. Recently, Rincon *et al.* (2002) reported morphological differences between the South African and Taiwanese strains of *C. plutellae*. In addition, these two strains have been found to be reproductively incompatible even though their DNA profiles (CO1 and ITS2) are identical (Rincon *et al.* 2002; B. Löhr, unpublished data). Could this be an indication that these two strains of *C. plutellae* are different species?

Azidah *et al.* (2000) examined, using morphological characteristics, specimens of *Diadegma* species associated with *P. xylostella* from several African countries, neighbouring Indian Ocean and South Atlantic Islands and concluded that they all belong to the same species- *D. mollipla*. Although the *Diadegma* species from Ethiopia was not studied by Azidah *et al.* (2000), it is assumed to be the same as *D. mollipla* (Aregay 2003). However, there is evidence that '*D. mollipla*' from Ethiopia is both morphologically and genetically different to *D. mollipla* strains from South Africa and Kenya, which are morphologically similar (Azidah *et al.* 2000), and it could be a different species altogether (Wagener *et al*. 2002). Although the South African and Kenyan strains of *D. mollipla* are morphologically the same, genetically they are different (Wagener *et al.* 2002). Very little is known about the efficacy of *D. mollipla* in controlling *P. xylostella*. It is the most abundant parasitoid of *P. xylostella* in East Africa (Nyambo & Pekke, 1995 in Akol *et al.* 2002) but in South Africa it is out-competed by *C. plutellae*, and is active mainly in spring (September-November) (Kfir 1997; Waladde *et al.* 2001; Mosiane *et al.* 2003).

Biological control programmes based on the use of parasitoids can be improved substantially through detailed studies of the biology and ecology of these natural enemies (Martínez-Castillo *et al.* 2002). Information on the biology of a parasitoid is important not only in formulating sound biological control programs but also in ensuring better chances of success when introduced elsewhere (Lim 1986). Also important in biological studies is how a given parasitoid performs in the presence of its ecological homologues. For this reason, it has

become common to compare biological characteristics of parasitoids (Chua & Ooi 1986; Talekar & Yang 1991; Wang & Keller 2002). Except for *C. plutellae* being the most abundant parasitoid of *P. xylostella* in South Africa (Kfir 1997; Waladde *et al.* 2001; Smith 2002; Mosiane *et al.* 2003), its biology has never been studied in the laboratory. *Diadegma mollipla* is synonymous to *D. stellenboschense* (Cameron, 1905), a parasitoid of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Azidah *et al.* 2000), and the only information available on the biology of this parasitoid, in the laboratory, was studied on *P. operculella* in South Africa (Broodryk 1971). Broodryk (1971) reared this parasitoid successfully on both *P. xylostella* and *P. operculella* in the laboratory.

1.3 Aims and objectives of the study

The aim of the study was to compare certain aspects of the biology of *C. plutellae* to *D. mollipla* on *P. xylostella* in the laboratory. This study addressed the following aspects: (i) preference for, and suitability of, different instars of *P. xylostella* to *C. plutellae* and *D. mollipla*, as well as their fecundity, and searching efficiency at different host and parasitoid densities; (ii) effects of temperature on parasitism of *P. xylostella* by *C. plutellae* and *D. mollipla*, and on their rates of development and emergence; and (iii) the role of parasitoids in controlling *P. xylostella* on unsprayed cabbage plots was assessed.

1.4 Introduction to study insects

Cotesia plutellae is probably the most widely distributed solitary larval endoparasitoid of *P. xylostella*. It is believed to have originated from Europe and later spread throughout the world with *P. xylostella* (Lim 1982; Waterhouse & Norris 1987; Talekar & Shelton 1993). It has been recorded in the Philippines and former U.S.S.R. (Telenga 1964), China (Liu *et al.* 2000), Malaysia (Ooi 1980), Taiwan (Talekar & Yang 1991), Thailand (Keinmeesuke, 1992 in Talekar 1997), Pakistan (Mustaque & Mohyuddin, 1987 in Shi *et al.* 2002), India (Simmonds, 1971 in Waterhouse & Norris 1987) and South Africa (Kfir 1997). Except for South Africa (Kfir 1998), *P. xylostella* is considered to be exotic in all the other countries (Talekar & Shelton 1993). It is therefore not clear what were the original hosts of *C. plutellae* before the introduction of *P. xylostella.* There are, however, reports of *C. plutellae* having a wide range of Lepidopteran hosts including other pests of brassicas (Waterhouse & Norris 1987; Cameron *et al.* 1997), but field data indicate that *P. xylostella* is its preferred host (Cameron *et al.* 1997). In South Africa, however, there are no reports of its alternative hosts. The importance of *C. plutellae* in the management of *P. xylostella* is based on two important facts.

Firstly, it has a relatively wide thermal tolerance (Talekar & Yang 1991). Secondly, high levels of parasitism by *C. plutellae* have been reported despite the heavy input of insecticides (Alam 1992; Ooi 1992; Syed *et al*. 1997; Waladde *et al.* 2001; Smith 2002). This last point is very important, as insecticides will continue to form part of a control strategy for *P. xylostella* in many areas. *Cotesia plutellae* has been used widely in biological control programs of *P. xylostella*, with introductions made into several countries around the world (Waterhouse & Norris 1987; Fitton & Walker 1992).

Diadegma mollipla (Holmgren), a solitary larval-pupal endoparasitoid, is an important parasitoid of *P. xylostella* in Africa and neighbouring Indian Ocean and South Atlantic Islands (Kfir 1997; Kfir & Thomas 2001; Waladde *et al.* 2001; Azidah *et al.* 2002; Nyambo & Pekke, 1995 in Akol *et al.* 2002). The first record of this parasitoid on *P. xylostella* was made in the 1930s in South Africa, but under the genus *Angitia* (Ullyett 1947). For a long time it remained an undescribed *Diadegma* (= *Angitia*) species, and was believed to be endemic to South Africa (Fitton & Walker 1992). Recently, it was reported that it is synonymous to *D. stellenboschense*, an important parasitoid of potato tuber moth, *P. operculella* (Azidah *et al.* 2000). Although it is reported to be the most abundant parasitoid in East Africa (Kenya, Uganda and Tanzania), it is comparatively rare in South Africa considering its contribution to total parasitism of *P. xylostella* (Kfir 2003).

CHAPTER 2:

Maintenance of *Plutella xylostella*, *Cotesia plutellae* and *Diadegma mollipla* cultures

2.1 *Plutella xylostella*

The culture of *P. xylostella* was established from larvae and pupae collected from the Brits-Agricultural Research Station (23°25'33"S, 27°76'67"E, altitude 1102) in the North West Province of South Africa in 1993 (Sereda *et al.* 1997). The insect culture was maintained in the laboratory at 22 ± 1°C (mean ± s.d.), 60 ± 5% RH and 16L: 8D photoperiod. The moths were allowed to oviposit on canola seedlings, *Brassica napus* L.*,* in rearing cages (43L X 30B X 33H cm) for 48 hours. The cages are made of wood except for the top and back, which are made of glass and gauze, respectively. The moths were provided with 10% sugar solution on a ball of cotton wool. After 48 hours, the seedlings were removed from the oviposition cages. *Plutella xylostella* eggs hatch in a day or two at 22°C, and to ensure a constant food supply, the developing larvae were transferred to fresh seedlings every second day. Some of the larvae were used for maintaining *C. plutellae* and *D. mollipla* cultures (see below) and for experiments, while others were reared through to adulthood to ensure a constant supply of *P. xylostella* larvae. New moth cages were initiated every week (each moth cage was kept for only seven days). About 12g of sterilised *P. xylostella* pupae was placed in a Petri dish and put inside a cage together with canola seedlings. Eclosing *P. xylostella* adults were used to repeat the process outlined above.

2.2 *Cotesia plutellae* and *Diadegma mollipla*

The culture of *C. plutellae* was established from cocoons and parasitised *P. xylostella* larvae collected from the Brits-Agricultural Research Station in 1993, and has been augmented with individuals collected from cabbage fields in the Gauteng Province over the years. *Diadegma mollipla* was established from cocoons and parasitised *P. xylostella* larvae collected from the University of Fort Hare's Research Farm in Alice (32°46'S, 26°50'E, altitude 540m) in the Eastern Cape Province of South Africa in 2000.

The parasitoids' cultures were maintained at 25 ± 1°C (mean ± s.d.), 65 ± 5% RH and 16L: 8D photoperiod, but in separate culture rooms. The parasitoids were kept in rearing cages similar to the ones used for *P. xylostella*, and were provided with honey, streaked thinly on the glass top of the cage, and water in glass vials (2.5 X 10cm) with a cotton wick at the mouth. *Cotesia plutellae* was reared on second instar larvae of *P. xylostella* and *D. mollipla* was reared on third instars. For both parasitoids, a ratio of 10 hosts: 1 female parasitoid was used, and a 2 males: 1 female parasitoid ratio was maintained in all the cages. Cabbage leaves

were spread on the floor of the cages with parasitoids, and *P. xylostella* larvae were introduced for parasitism. The larvae were exposed to the parasitoids for 48 hours. After this period, the larvae were removed and reared further on cabbage leaves in Perspex dishes (11 X 23cm). In order to prevent water condensation in the dishes, a layer of paper towel was placed underneath the cabbage leaves. To prevent the larvae from escaping, the dishes were closed with gauze held tightly with rubber bands. The larvae were provided with fresh leaves every other day until they pupated and/or parasitoid cocoons formed. Parasitoid cocoons were collected and placed in honey jars (7 X 14cm), and thin streaks of honey were made on the inside walls of the jars. Eclosing parasitoids were used to maintain the parasitoid cultures in the manner described above, while others were used for experiments.

CHAPTER 3:

Host instar preference, fecundity and searching efficiency of *Cotesia plutellae* and *Diadegma mollipla*

3.1 General introduction

Biological control of insect pests using parasitoids can be improved substantially through detailed studies of the biology and ecology of these natural enemies (Martínez-Castillo *et al*. 2002). In this view, it is essential to examine and estimate closely the real potential and/or limitations of these biological control agents individually. Not only is this information necessary in formulating sound biological control programs but also in ensuring better chances of success (Lim 1986). Because an insect pest can serve as a host to a complex of parasitoid species and that these natural enemies may compete for the same host stage, an understanding of the interaction between these natural enemies is very important (Chua & Ooi 1986; Talekar & Yang 1991; Wang & Keller 2002). In addition, biological aspects such as fecundity and searching efficiency of females can reveal the competitiveness of parasitoids.

The biology of *Cotesia plutellae* has been studied extensively in Southeast Asia (Lim 1982; Chua & Ooi 1986; Talekar & Yang 1991; Lim & Mohamed 1992; Kawagushi & Tanaka 1999; Shi & Liu 1999). Although phenological information is available on *C. plutellae* in South Africa (Kfir 1997; Waladde *et al.* 2001; Smith 2002; Mosiane *et al.* 2003), its biology has never been studied in the laboratory. Rincon *et al*. (2002) discovered morphological differences between the South African and Taiwanese strains of *C. plutellae*. In addition, it was reported in two independent studies that the South African and Taiwanese strains of *C. plutellae* are reproductively incompatible (Rincon *et al.* 2002; B. Löhr, unpublished data). This evidence, together with the differential ability of *C. plutellae* to control *P. xylostella* in South Africa and Southeast Asia, has shown the need to study the biology of the South African strain of *C. plutellae* and, where possible, to compare its biological characteristics to that of the Southeast Asian strain.

The biology of *D. mollipla* has never been studied on *P. xylostella* in the laboratory, and the only information on its biology was studied on *P. operculella* in South Africa (Broodryk 1971).

3.2 General materials and methods

The following procedures were followed in each of the following experiments, unless it is stated otherwise.

1. All experiments were carried out in the laboratory at 25 ± 1°C (mean ± s.d.), 60 ± 5% RH and 16L: 8D photoperiod. *Plutella xylostella* larvae were exposed for parasitism in Perspex buckets (18 X 23cm) (see Figure 3.1).
2. Only newly emerged (<24 hours old) parasitoids were used for the experiments and to ensure mating and fertilisation, each female was confined with four males in a glass vial (2.5 X 10cm) for 24 hours.
3. The parasitoids were provided with honey streaked thinly on the sides of the buckets.
4. Each experiment had 35 replicates.

3.3. Preference for, and suitability of, various instars of *Plutella xylostella*

3.3.1 Introduction

Host stage is an important ecological variable that may influence the survival of immature stages, rate of development, sex ratio, size and fecundity of parasitoids (Godfray 1994; Islam & Copland 1997; Fidgen *et al.* 2000). Because searching parasitoids often encounter hosts of different developmental stages within a patch, and because host stages potentially vulnerable to attack may differ in their productivity (Alphen & Jervis 1996), a female parasitoid has to choose which hosts to attack and which to ignore (Rivero 2000). Parasitoids have evolved behavioural, ecological and physiological adaptations that enable them to discriminate among, and to differentially utilise, their hosts (Karamaouna & Copland 2000a). Due to selectivity of parasitoids for the hosts they attack (host selection behaviour), the relative frequency of host instar parasitised compared to the frequency of host instars present is called host instar preference (Karamaouna & Copland 2000b). Some studies have shown that decisions on host instar selection by parasitoids are dynamic (i.e. not all host instars above and below certain thresholds of acceptability, specific to each parasitoid, are always accepted or rejected), and that host acceptability or rejection also depends on the physiological and information state of the foraging insect (Rivero 2000; Powell & Poppy 2001).

Because most hymenopterous parasitoids are arrhenotokous [i.e. males develop parthenogenetically from unfertilised (haploid) eggs while females develop from fertilised (diploid) eggs], a female makes oviposition decisions based on the host instar encountered, and also has to decide the sex of each offspring (King 1993; Godfray 1994). Parasitoids, in

general, determine the quality of a host by its size, with large hosts believed to contain more resources than small hosts (Godfray 1994; Islam & Copland 1997). Because of the relationship between the size of a parasitoid and that of the host in which it developed, and because body size affects female fitness (longevity, fecundity and searching efficiency) more than male mating success, mothers are selected to lay the sex of the offspring that will benefit the most from the host size encountered (Murdorch *et al.* 1992; Godfray 1994; Alphen & Jervis 1996). Generally, male eggs are laid in smaller or poor quality hosts, while female eggs are laid in larger or better quality hosts (Murdorch *et al.* 1992). However, such differential sex allocation also depends on the relative distribution of the host sizes (Bernal *et al.* 1998; Heinz 1998). In addition, information on host instar preference may determine whether two parasitoids that share the same host instars are able to coexist, or if one of the two species will become extinct if they are released together (Wang & Keller 2002). However, the exclusion of either species would depend on niche width of each parasitoid and the degree of niche overlap between the parasitoids, as well as on parameters such as host searching efficiency, fecundity and female production per female (Huffaker & Laing 1972; Karamaouna & Copland 2000b).

In the study reported here I investigated the preference for different instars of *P. xylostella* by *C. plutellae* and *D. mollipla*, and the suitability of these host instars on rate of development, emergence, and female production per female for both parasitoids.

3.3.2 Materials and Methods

The host instar preference experiment was divided into two parts, *no-choice* and *choice* tests. *No-choice test*: 30 *P. xylostella* larvae of either second, third or fourth instars were placed on cabbage leaves in a Perspex bucket (Figure 3.1) and exposed to the parasitoids. To prevent the larvae from escaping, the buckets were closed with gauze held tightly with rubber bands. *Choice test*: 30 *P. xylostella* larvae (10 of each instar) were exposed for parasitism simultaneously in the manner described above.

For both tests, a mated but naive (never encountered a suitable host before) one-day-old female was released into each bucket with hosts for 24 hours. After this period, the parasitoids were removed. The larvae were reared further on sections of cabbage leaves in honey jars (7 X 14 cm). To prevent water condensation in the jars, a piece of paper towel was placed underneath the leaf, and the jar was closed with gauze held tightly with rubber bands. For the

choice test, however, the larvae were sorted according to their respective host stages immediately after the removal of parasitoids, and reared separately in honey jars. The larvae were provided with sections of fresh cabbage leaves every second day until they pupated and/or parasitoid cocoons formed. *Cotesia plutellae* cocoons or *P. xylostella* pupae containing *D. mollipla* were placed in-groups of five in Petri dishes. Each Petri dish was tightly closed with rubber bands. For each host stage, the number of hosts parasitised and unparasitised, rates of development of parasitoid offspring, eclosion level, and the sex ratio of the emerging parasitoids were recorded. Hosts that died of unknown causes were also recorded but these were excluded from the calculations.

Choice test by observation: this test was designed to supplement the *choice test* described above by observing the frequency of attacks on various instars (2^{nd}, 3^{rd} and 4^{th}) of *P. xylostella* by *C. plutellae* and *D. mollipla*. Nine hosts (3 of each instar) were exposed for parasitism simultaneously in a Petri dish. A mated but naive one-day-old female was introduced into each Petri dish. The number of times that each host instar was attacked by the parasitoid was recorded, and the observations were conducted for one hour. The oviposition behaviour of the parasitoids was also observed.

3.3.3 Data analysis

The data on host instar preference experiments (*Choice test, No-choice test* and *Choice test by observation*) by *C. plutellae* and *D. mollipla* were analysed using a Chi-squared (χ^2) test. A Chi-squared test is generally used to test for agreement between observation and hypothesis. The usual hypothesis is that all expected values are the same. When a sample of individuals is classified according to two attributes the result is a two-way frequency table known as a row-by-column (*r x c*) contingency table (Snedecor & Cochran 1980). The Chi-squared row-by-column test is useful in determining significant differences between the two independent attributes. This test, however, has certain limitations, for instance, the rows may not have an expected frequency of less than 3 (Siegel 1956). The contingency table used in this study was made up of three rows (i.e. host instars) and two columns (i.e. parasitised and unparasitised hosts).

To determine the effect of host instar on the duration of development, emergence and proportion of females in the parasitoids' progeny, the means were subjected to Analyses of Variance (ANOVA). The purpose of an ANOVA is to determine if there are any significant

differences between means of samples. ANOVA should be followed by a multiple range test to determine where the significant differences are (Elliot 1983). In this case, ANOVA was followed by a Fisher's Protected Least Significant Difference test. Before analysis, data were subjected to a Bartlett test for homogeneity and Watson test for normality. All the data (both Chi-squared test and ANOVA) were analysed using the statistical program Genstat 5 (Genstat for Windows 2000).

3.3.4 Results

No-choice test

Cotesia plutellae showed a higher preference for third instar hosts than for second and fourth instars (Fig. 3.2A). Comparisons of the means indicated that preference for third instar hosts was significantly higher than for second ($\chi^2 = 8.394$, d.f. = 1, $P < 0.0001$) and fourth instars ($\chi^2 = 160.071$, d.f. = 1, $P < 0.0001$). In addition, *C. plutellae* parasitised a significantly higher proportion of second instar hosts than fourth instars ($\chi^2 = 100.006$, d.f. = 1, $P < 0.0001$). *Diadegma mollipla* also showed a higher preference for second instar hosts than third and fourth instars (Fig. 3.2B). Comparisons of means indicated that preference for second instar hosts was significantly higher than for third ($\chi^2 = 16.072$, d.f. = 1, $P < 0.001$) and fourth instars ($\chi^2 = 167.411$, d.f. = 1, $P < 0.0001$). Preference for third instar hosts was also significantly higher than for fourth instars ($\chi^2 = 78.051$, d.f. = 1, $P < 0.0001$).

The duration of development of progeny for both parasitoid species appeared to be influenced by the size of the host at the time of oviposition (Tables 3.1 and 3.2). No significant differences were observed in the duration of development, in all three *P. xylostella* larval instars, from the egg-pupal stage in *C. plutellae* ($F = 1.01$ NS, d.f. = 2, 87, $P = 0.369$) (Table 3.1). However, significant differences were observed in the duration of development from the pupal-adult stage ($F = 29.93$, d.f. = 2, 87, $P < 0.001$), and these were between individuals that developed in fourth instar hosts than in either second or third instars, which are not significantly different (Table 3.1). As a consequence the generation time was significantly shorter for individuals that developed in fourth instar hosts than those that developed in second or third instars ($F = 12.43$, d.f. = 2, 87, $P < 0.001$) (Table 3.1). Significant differences in the duration of development (egg-pupal stage) were observed in *D. mollipla* ($F = 5.93$, d.f. = 2, 87, $P = 0.004$), and these were between second and fourth instars but not between third and fourth or second instars (Table 3.2.). Significant differences were also detected in the

duration of development (pupa-adult) between host stages (F = 12.27, d.f. = 2,87, $P < 0.001$), and the between second and fourth instar hosts, and between third and fourth instars, none between second and third instars (Table 3.2). As a consequence of all the above mentioned differences in the duration of development, the generation time (egg-pupal development) was also significant (F = 9.38, d.f. = 2, 87, $P < 0.001$) (Table 3.2).

The proportion of *C. plutellae* individuals that completed development was significantly different between host stages (F = 38.62, d.f. = 2, 87, $P < 0.001$), and eclosions were higher in second and third instar hosts than in fourth instars (Table 3.1). Eclosion of adult parasitoids was also significantly different between *P. xylostella* instars (F = 32.20, d.f. = 2, 87, $P < 0.001$), and it was higher in third instar hosts compared to either second or fourth instars (Table 3.2). There were significant differences in the proportions of female parasitoids between *P. xylostella* larval instars (*C. plutellae*: F = 15.49, d.f. = 2, 87, $P < 0.001$; *D. mollipla*: F = 28.71, d.f. = 2, 87, $P < 0.001$), and for both parasitoid species, the younger the host was at the time of oviposition, the higher the proportion of females in progeny (Tables 3.1 and 3.2).

Choice test

In contrast to the *no-choice test* experiment, in this experiment *C. plutellae* showed a higher preference for second instar hosts than third and fourth instars (Fig. 3.3A). Comparisons of the means indicated that preference for second instar hosts by *C. plutellae* was significantly higher than that of third (χ^2 = 22.157, d.f. = 1, $P < 0.0001$) and fourth instars (χ^2 = 281.128, d.f. = 1, $P < 0.0001$). In addition, *C. plutellae* parasitised a significantly higher proportion of third instar hosts than fourth instars (χ^2 = 176.008, d.f. = 1, $P < 0.0001$). *Diadegma mollipla* appeared to have a similar preference for both second and third instar hosts over fourth instars (Fig. 3.3B). Comparisons of means did not show any significant differences in preference between second and third instar hosts (F = 0.036 NS, d.f. = 1, $P < 0.0001$), but there were significant differences in preference for second instar hosts than fourth instars (χ^2 = 29.511, d.f. = 1, $P < 0.0001$) and for third instar hosts than fourth instars (χ^2 = 40.664, d.f. = 1, $P < 0.0001$).

In accordance with the results of the *no-choice test* above, where it was shown that the younger the host was at the time of oviposition the higher the proportion of females that

emerge, in this experiment *C. plutellae* laid more female eggs in second instars hosts than in third and fourth instar hosts (Table 3.3). *Diadegma mollipla*, on the other hand, laid significantly more female eggs in fourth instar hosts than in second and third instar hosts (Table 3.3).

Choice test by observation

Significant differences in the attack rates between the different *P. xylostella* larval instars were detected in *C. plutellae* (χ^2 = 73.812; d.f. = 2; $P < 0.0001$), and it attacked more of second instar hosts than third or fourth instars (Fig. 3.4A). Although *D. mollipla* attacked a slightly higher proportion of second and third instar hosts than fourth instars (Fig. 3.4B), No significant differences were observed in the attack rate of the different *P. xylostella* larval instars (χ^2 = 2.939; d.f. = 2; P = 0.2280). However, it needs to be stressed that a female does not necessarily lay an egg in each and every host that it probes.

Observations of the oviposition behaviour of *C. plutellae* and *D. mollipla* showed that when a female wasp attacked second and third instar *P. xylostella* larvae, the wasp and the host twisted around each other for a couple of seconds before the two insects separated. It is therefore presumed that the parasitoid inserts its ovipositor and lays an egg at this time. Attempting parasitism on fourth instars, on the other hand, showed that although the ovipositor of an ovipositing female made contact with the host it was unable to twist around with the host as it does with second and third instars, and this was more evident in *C. plutellae.* This is probably due to the fact that fourth instars are too large and too active to be easily seized by *C. plutellae. Diadegma mollipla* displayed different oviposition behaviour on fourth instar hosts: instead of pinning down the larva with its ovipositor and twisting with it, it bent its abdomen and tried to parasitise it in much the same way as an aphid parasitoid. In many instances, females of both parasitoid species were observed to avoid most of the fourth instars they encountered, particularly after they had had a previous experience in an encounter with a fourth instar larva.

Fig. 3. 1. The experimental equipment used to expose *Plutella xylostella* larvae to *Cotesia plutellae* and *Diadegma mollipla* females for parasitism

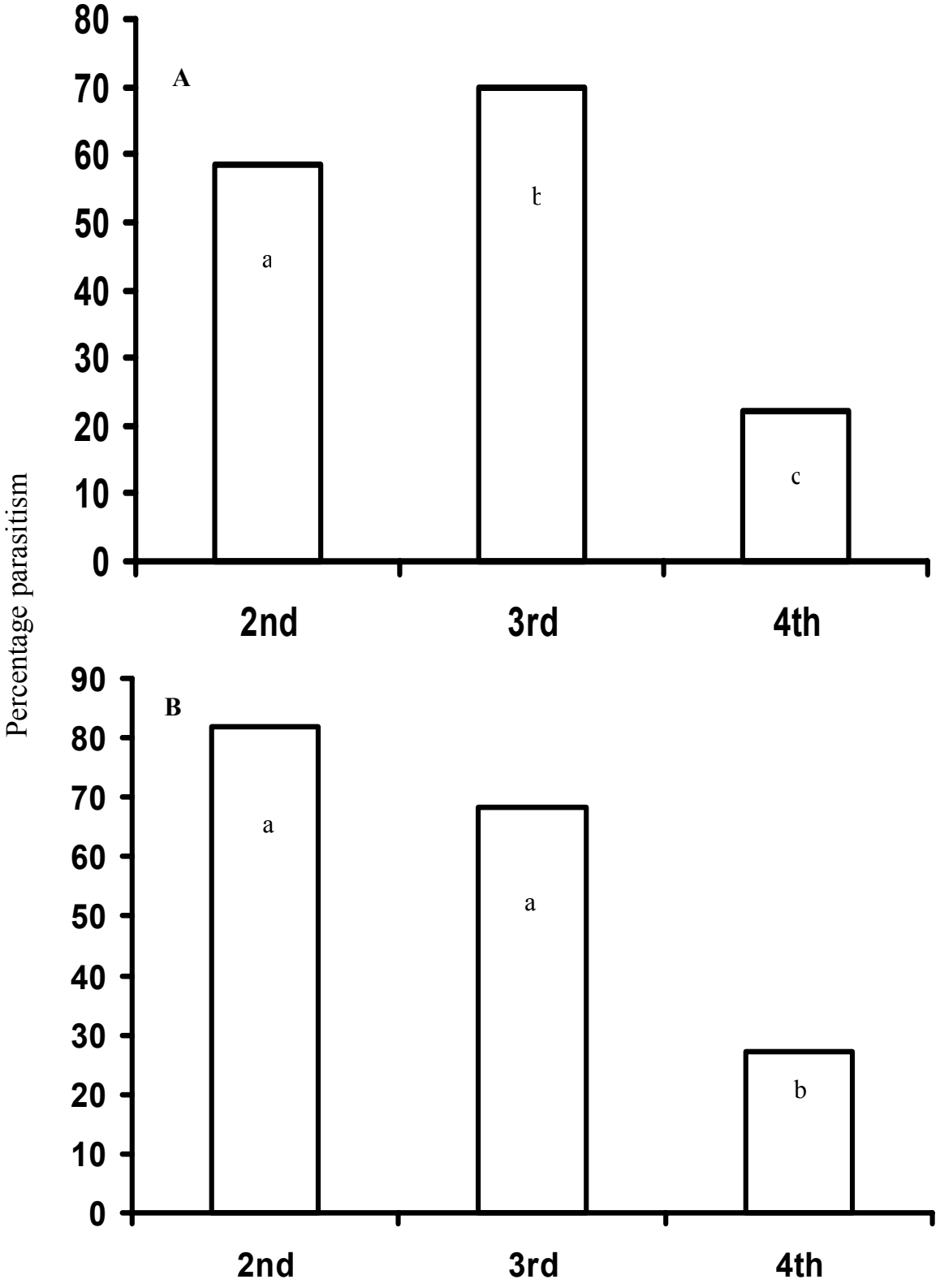

Fig 3.2. Parasitism of various instars of *Plutella xylostella* exposed separately (*no-choice test*) to *Cotesia plutellae* (**A**) and *Diadegma mollipla* (**B**). Host instars with the same letter are no significantly different.

Table 3.1. Suitability of various instars of *Plutella xylostella* to *Cotesia plutellae* (*no-choice test*). Values in the same column followed by the same letter are not significantly different.

Host instar	n	Rate of development (mean ± s.e.) in days			% of females in progeny (mean ± s.e.)	% emergence (mean ± s.e.)
		egg-pupa	pupa-adult	egg-adult		
Second	244	7.3 ± 0.05a[1]	6.73 ± 0.1a[2]	13.95 ± 0.11a[3]	58.92 ± 5.78a[4]	66.22 ± 3.96a[5]
Third	221	7.56 ± 0.11a	6.65 ± 0.25a	14.28 ± 0.19a	37.33 ± 6.56b	74.26 ± 4.11a
Fourth	86	7.82 ± 0.43a	3.68 ± 0.48b	11.5 ± 0.71b	27.38 ± 8.86b	47.24 ± 7.02b

LSD values ($P > 0.05$; Fisher's Protected LSD test): 1 = 0.723; 2 = 0.892; 3 = 1.212; 4 = 18.36; 5 = 14. 26

Table 3.2. Suitability of various instars of *Plutella xylostella* to *Diadegma mollipla* (*no-choice test*). Values in the same column followed by the same letter are not significantly different.

Host instar	n	Rate of development (mean ± s.e.) in days			% of females in progeny (mean ± s.e.)	% emergence (mean ± s.e.)
		Egg-pupa	pupa-adult	egg-adult		
Second	317	6.42 ± 0.24a[1]	8.17 ± 0.32a[2]	14.58 ± 0.51a[3]	60.19 ± 5.45a[4]	55.14 ± 4.43a[5]
Third	196	5.22 ± 0.44ab	7.49 ± 0.7a	12.71 ± 1.11a	38.49 ± 7.18b	67.81 ± 4.95b
Fourth	54	4.15 ± 1b	4.32 ± 0.67b	8.47 ± 1.29b	34.0 ± 12.18b	49.88 ± 6.96a

LSD values ($P > 0.05$, Fisher's Protected LSD test): 1 = 1.31; 2 = 1.65; 3 = 2.876; 4 = 18.19; 5 = 12.61

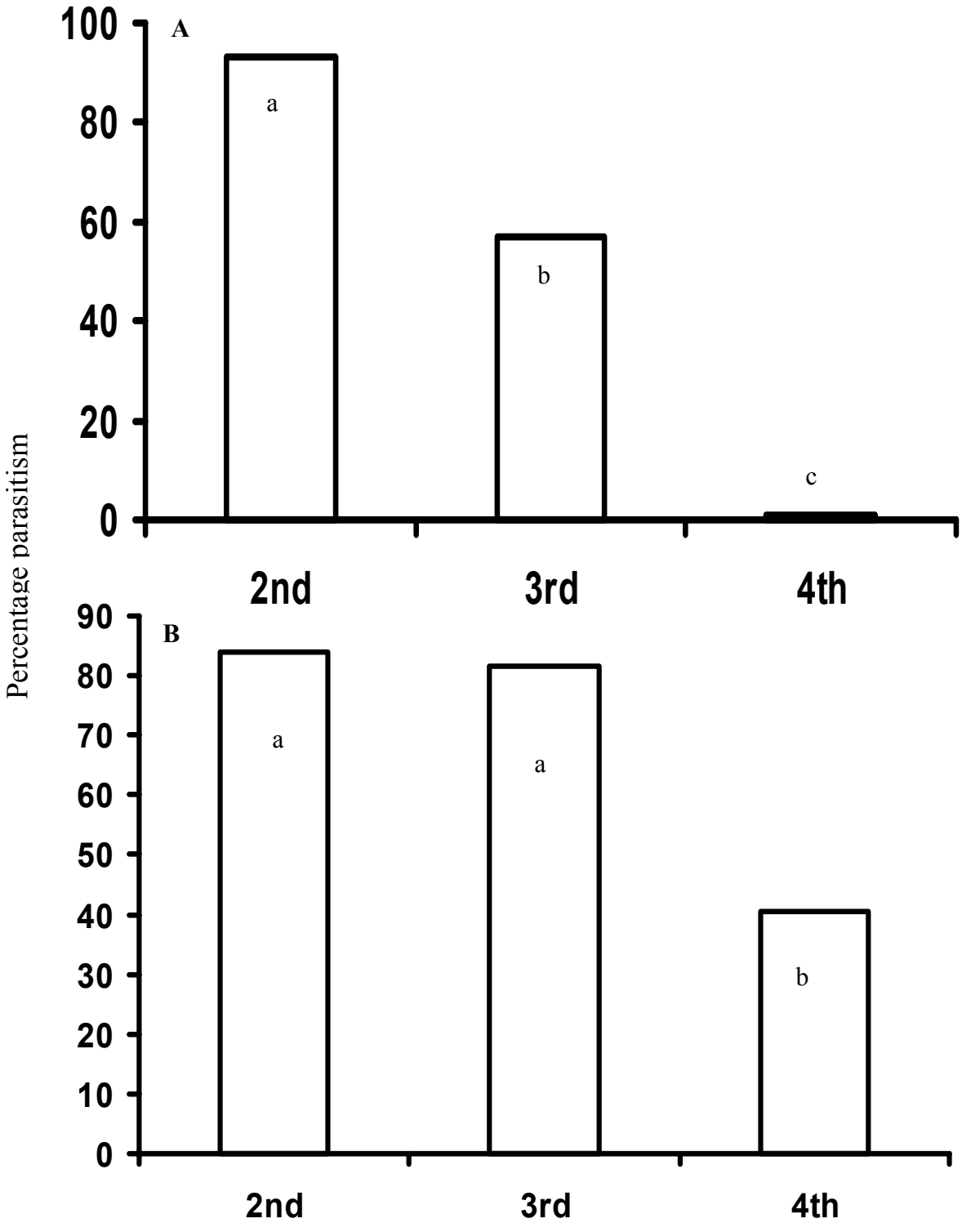

Fig. 3.3. Preference for various *Plutella xylostella* instars exposed simultaneously (*choice test*) to *Cotesia plutellae* (**A**) and *Diadegma mollipla* (**B**). Host stages with the same letter are not significantly different.

Table 3.3. Effect of host instar at the time of oviposition on the sex ratio of parasitoid progeny

Host instar	*Diadegma mollipla*		*Cotesia plutellae*	
	n	% females	n	% females
Second	34	26.47	28	32.14
Third	59	23.73	37	21.62
Fourth	11	54.55	1	0

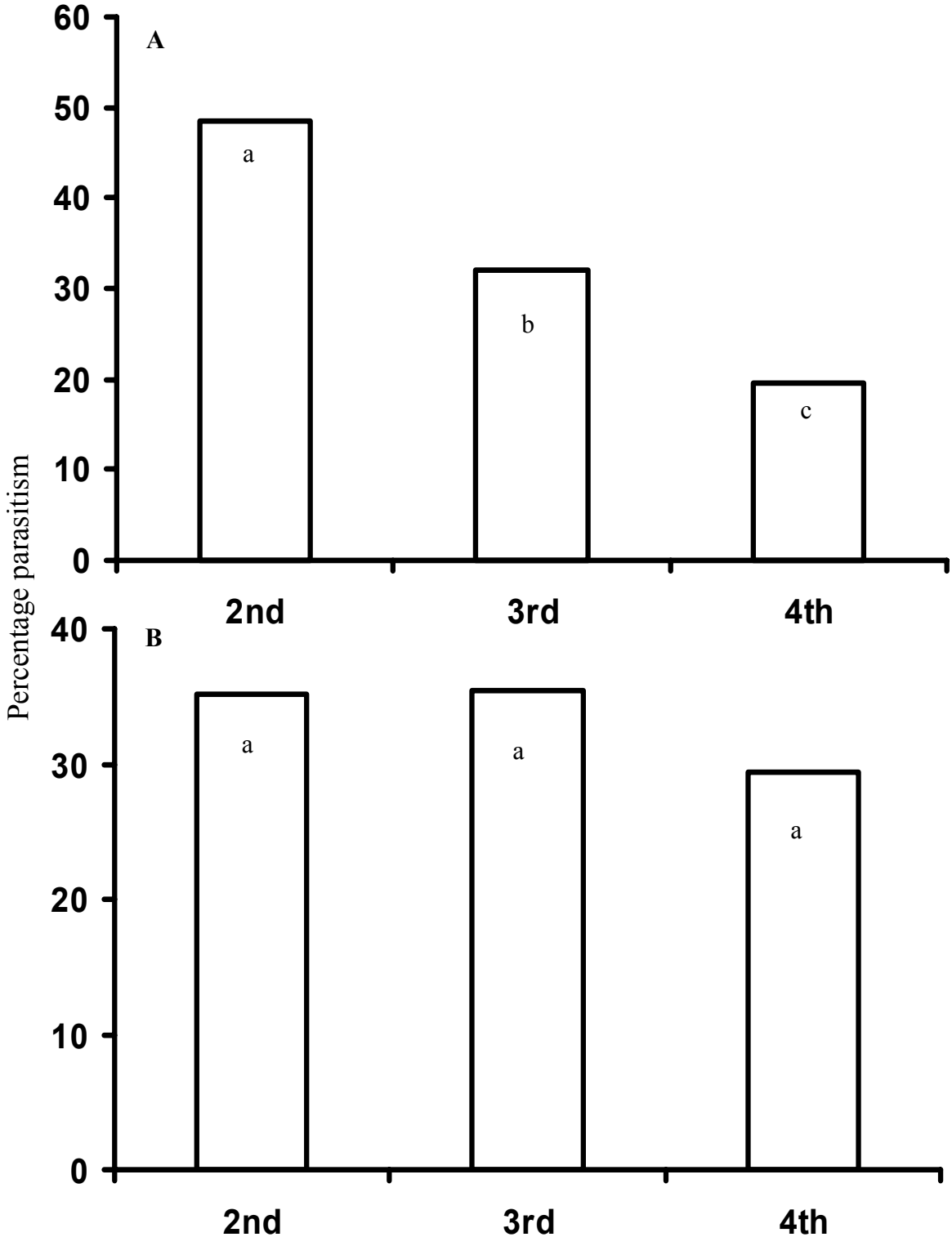

Fig. 3.4 Frequency of attacks on different *Plutella xylostella* instars (*choice test by observation*) by *Cotesia plutellae* (**A**) and *Diadegma mollipla* (**B**). Host instars with a same letter are not significantly different.

3.4 Fecundity

3.4.1 Introduction

Reproduction is one of the important factors that determine the rate of increase of an animal's population. Thus, the reproductive capacity of individuals can be used to estimate the rate of increase at the population level. The two parameters used to measure the reproductive capacity of insects are fecundity and fertility. *Fecundity* refers to the animal's reproductive output (i.e. number of eggs produced or laid) whereas *fertility* refers only to the number of viable progeny that ensue (Jervis & Copland 1996). Because not all of the eggs laid will give rise to adult offspring, from the standpoint of population dynamics, fertility appears to be the most important parameter because it gives an estimate of the number of individuals entering the next generation (Jervis & Copland 1996). However, take a scenario where a parasitoid larva develops to a pupal stage, killing its host in the process, but fails to give rise to an adult parasitoid. Even though no parasitoid offspring emerged, such host mortality contributes to a reduction in the pest's population. This leads to a concept called *realised fecundity* (Mills & Kuhlman 2000), which can be taken to refer to the number of parasitoid cocoons formed, and this differs from fertility that refers only to the number of viable progeny that ensue from these cocoons. For convenience, *realised fecundity* will be taken to be synonymous with *fecundity*.

3.4.2 Materials and methods

To determine whether *C. plutellae* and *D. mollipla* are able to attack hosts from eclosion until death, 30 newly emerged (<24 hours old) females were each confined with four males in glass vials (10 X 2.5cm) for four hours in order to ensure mating and fertilisation. A female was regarded as fertilised only if it was seen mating, and only such females were used in this experiment. Copulation takes a few seconds in *C. plutellae* whereas in *D. mollipla* it can last more than 20 minutes. Thirty third instar *P. xylostella* larvae were placed in a Perspex bucket, in a similar manner as described for the host instar preference experiment above. One mated female was introduced into each of the buckets with hosts for 24 hours. After this period, the females were transferred into buckets with fresh hosts for another 24 hours. This process was repeated until the death of the females. Larvae exposed for parasitism were transferred to honey jars, and reared through on sections of cabbage leaves until pupae and/or parasitoid cocoons formed. The age at which females start parasitising hosts, the number of progeny produced per day, and the total number of progeny per female (where progeny refers to the number of cocoons produced) were all recorded.

3.4.3 Results

Both *C. plutellae* and *D. mollipla* were able to parasitise hosts on the day of eclosion (Figs. 3.5A and 3.5B). Therefore, they are pro-ovigenic [i.e. they have a capacity to store a full complement of mature eggs in the ovaries or oviducts, and complete oogenesis either before or very soon after eclosion using food reserves built up during larval life (Mills & Kuhlman 2000)]. Both parasitoid attacked hosts for almost all of their life span, but fecundity decreased progressively as the females aged (Figs. 3.5A and 3.5B). For *C. plutellae*, fecundity was highest (about 10 cocoons per female per day) during the first 2 days after eclosion whereas for *D. mollipla* it peaked at about 11 cocoons per female per day during the first 5 days of eclosion. On average, a female *C. plutellae* produced a total of 42.13 ± 12.2 (mean ± s.d.) progeny in a life span of 5.23 ± 2.7 days, whereas *D. mollipla* had a total fecundity of 82.57 ± 32.87 progeny per female in a life span of 7.13 ± 3.69 days.

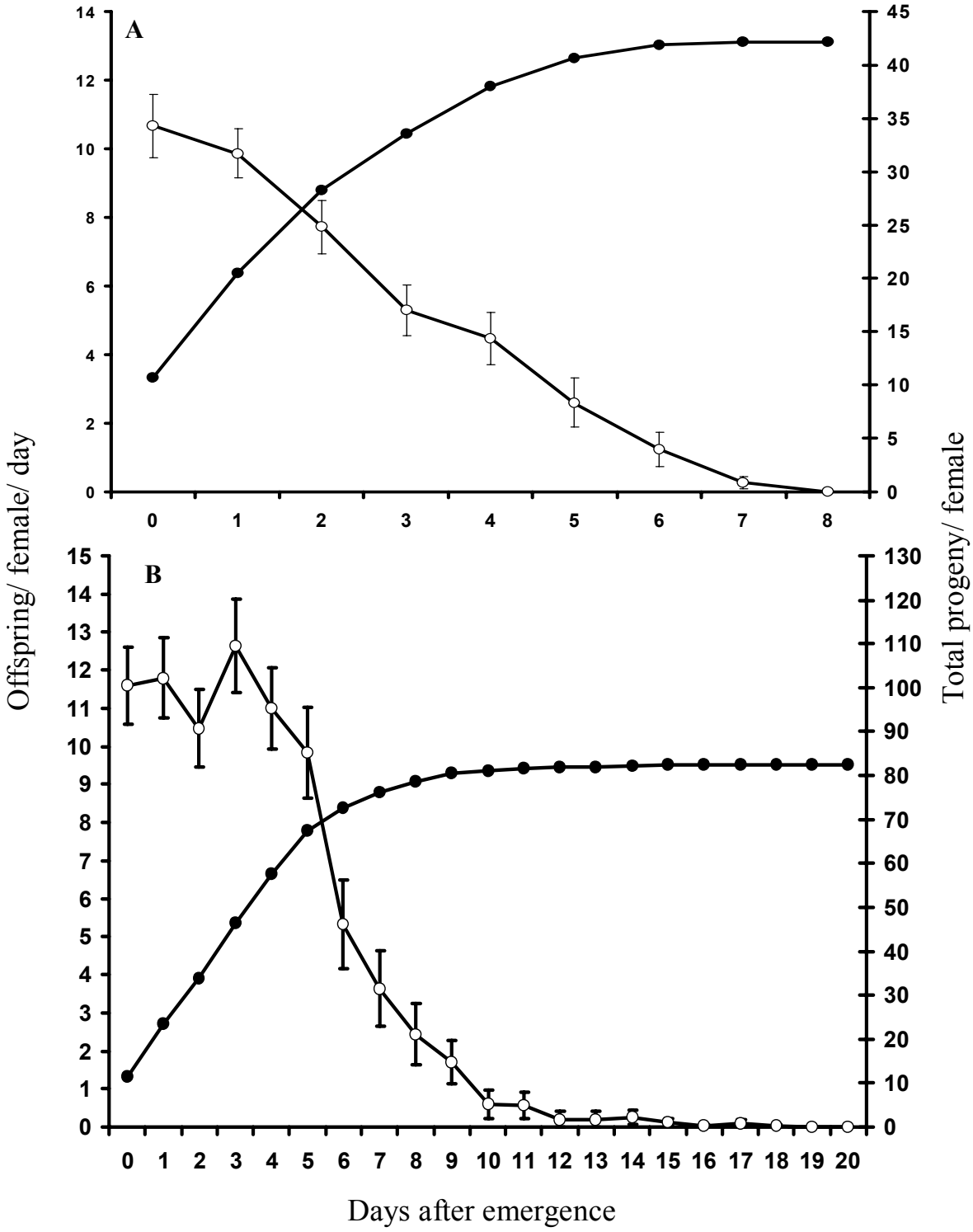

Fig. 3.5 Fecundity (n = 30) of *Cotesia plutellae* (**A**) and *Diadegma mollipla* (**B**). Open circles represent the number of offspring produced per female per day; solid circles represent the total number of offspring per female

3.5 Searching efficiency of *Cotesia plutellae* and *Diadegma mollipla*

3.5.1 Introduction

Parasitoids search for hosts in order to leave as many offspring as possible to survive and reproduce in the next generation (Powell & Poppy 2001). In solitary parasitoids, there is generally a direct link between the rate of host encounter and lifetime offspring production, therefore a female's searching efficiency (i.e. its ability to find and parasitise hosts) is important in determining its efficacy in regulating pest population dynamics (Vet 2001). At the inter-specific level, a parasitoid species with a higher searching efficiency will have a competitive advantage at low host densities. The searching efficiency of a parasitoid is affected by (i) its response to host density, (ii) its response to other parasitoids, and (iii) the distribution of searching females in relation to host distribution (Hassell & Rogers 1972).

Host density: Holling (1959, in Hassell & Rogers 1972) pointed out that there is always a certain time interval between parasitism and search being resumed, a period he termed *handling time*. This handling time is believed to progressively reduce the time available for searching, as more and more hosts are encountered (Hassell & Rogers 1972; Rivero 2000). **Parasitoid density**: As parasitoids aggregate in patches of high host density, it is increasingly likely that they will encounter each other while searching for hosts, and such encounters between searching parasitoids may result in time-wasting (see below); time which could have been used to parasitise or search for hosts (Hassell 1978). Superparasitism, the deposition of eggs in or on a host that has already been parasitised by a conspecific female, generally results in reduction in the survivorship of larvae or in the size, longevity and fecundity of ensuing adults (Potting 1995). In this respect, parasitised hosts are often rendered unsuitable for exploitation by a second parasitoid, so that, as the number of unparasitised hosts gets depleted, competition intensifies among searching females (Hassell 1978). Foraging theory predicts that the optimal response to encountering an already parasitised host is often rejection of that host (Potting 1995; Rivero 2000). Other studies have shown that females react to competition in various forms- some fight or delay searching for hosts upon encountering a competitor, as more of these occur at high parasitoid densities, the result is a reduced oviposition rate per female (Alphen & Jervis 1996). Other behaviours influenced by high parasitoid densities include decisions on superparasitism, sex ratio, clutch size, brood or patch guarding, or increased rate of dispersal of the females (Hassell 1978; Heinz 1998; Visser *et al.* 1999; Alphen *et al.* 2003). Comparative studies of the searching efficiency of two parasitoid species that share the same ecological niche (e.g. hosts and host stages) can reveal the extent

of the interaction between them, differences in intrinsic competitiveness and the capacity for co-existence (Wang & Keller 2002).

The aim of this study was to compare the searching efficiency of *C. plutellae* to *D. mollipla* at different host and parasitoid densities.

3.5.2 Materials and methods

For a successful pest management program, information on host searching efficiency (*a*), mutual interference (*m*), and killing power (*k*) of parasitoid species is important (Kumar & Tripathi 1984). Host searching efficiency (to be referred to as *area of discovery*) was calculated using Hassell's (1971) formula:

$$a = \frac{1}{p} \log e \frac{N_i}{N_f}$$

where P is the parasitoid density, and N_i and N_f are the initial and final (unparasitised) host densities, respectively. The relationship between a and P is described by Hassell & Varley's (1969) formula:

$$a = QP^{-m}$$

where Q is the quest constant (i.e. area of discovery when only one female is searching), and m is the interference constant between searching parasitoids. Expressed in logarithms, the equation becomes linear, and m is the slope of regression of *log a* on *log P*:

$$\log a = \log Q - m \log P$$

The killing power of the parasitoid, K, is calculated using the formula given in Ooi (1980):

$$K = \log 10 \frac{N_i}{N_f}$$

This experiment was divided into two parts: the first part, investigated the functional response of the parasitoids; and the second part, the magnitude of interference (mutual interference) between searching females.

To measure the functional response of *C. plutellae* and *D. mollipla*, host densities of 20, 40, 60, 80 and 100 hosts per Perspex bucket were set up, and each host density was exposed to a one-day-old mated but naive female. To measure mutual interference, parasitoid densities of 1, 2, 4, 6, 8 and 10 one-day-old mated but naive females per bucket were set up, and each parasitoid density was provided with 100 hosts. Each host or parasitoid density had five replicates. Third instar *P. xylostella* larvae were used in both experiments. The larvae were exposed for parasitism for 24 hours. After this period, the parasitoids were removed and the hosts were reared further on sections of fresh cabbage leaves in Perspex buckets until they pupated and/or parasitoid cocoons formed. *Cotesia plutellae* cocoons or *P. xylostella* pupae containing *D. mollipla* were placed in-groups of five in Petri dishes. Each Petri dish was tightly closed with rubber bands. The number of hosts parasitised and unparasitised were recorded, as well as the sex ratio of eclosing parasitoids. Hosts that died of unknown causes were also recorded; some of these were excluded from the calculations while others were included (see below).

The exposure of a limited number of hosts to a high density of parasitoids can lead to superparasitism, which as a consequence can result in high mortality of the hosts. Further, observations made in the *choice test by observation* experiment revealed that a *P. xylostella* larva that is probed several times by a parasitoid normally died. It is possible therefore that in any given experiment some of the larvae will die from multiple probing. In order to quantify the contribution of multiple probing or superparasitism to the total mortality of *P. xylostella* larvae due to unknown causes, controls (i.e. without parasitoids) were set up for each of the test densities of 20, 40, 60, 80 and 100 larvae per bucket. Each larval density had five replicates. The larvae were reared from the second instar on cabbage leaves in Perspex buckets in the manner as described above. The number of larvae that died and those that survived to the pupal stage were recorded. The number of moths that emerged from these pupae was also recorded, as well as the number of pupae that failed to yield any moths. Since *C. plutellae* is a larval parasitoid, the larvae that died accounted for natural mortality in larvae exposed to *C. plutellae*. For *D. mollipla*, a larval-pupal parasitoid, natural mortality comprised both larvae and pupae that died in the controls. Because mortality of *P. xylostella* larvae exposed to parasitism was higher than in the control with a corresponding initial host density, the difference in mortality was attributed to parasitism (Table 3.4). The difference in mortality between hosts exposed to parasitism and those in the control for each host density was added to the number of parasitised hosts.

3.5.3 Data analysis

To investigate the effects of different host and parasitoid densities on the searching efficiency and killing power of *C. plutellae* and *D. mollipla*, the means were subjected to an ANOVA followed by a Fisher's Protected Least Significant Difference test. Before the analysis, the data were subjected to a Bartlett test for homogeneity and Watson test for normality. Further, in order to establish which of the two parasitoid species has higher values for searching efficiency and killing power, the means were subjected to a paired-sample t-test. All the data were analysed using the statistical program Genstat 5 (Genstat for Windows 2000).

3.5.4 Results

The increase in host density resulted in reduction in the area of discovery (*a*) and killing power (*K*) for both *C. plutellae* and *D. mollipla* (Table 3.5). The searching efficiency of *C. plutellae* declined significantly with increasing host density (F = 16.11, d.f. = 4,20, $P <$ 0.001), and the differences were significant only between the 20-host density and all the other host densities (Table 3.5). Significant differences in searching efficiency were also detected for *D. mollipla* as the host density is increased (F = 9.67, d.f. = 4,20, $P < 0.001$), and these were also between the 20-host density and all the other host densities (Table 3.5). No significant differences were observed when the searching efficiencies of the two parasitoid species were compared (t = -1.42 NS, d.f. = 48, P = 0.148). Significant differences were also observed in killing power of the two parasitoid species when the host density is increased (*C. plutellae*: F = 16.12, d.f. = 4, 20, $P < 0.001$; *D. mollipla*: F = 9.67, d.f. = 4, 20, $P < 0.001$), and these differences were only observed between the 20-host density and all the other host densities (Table 3.5). An increase in parasitoid density resulted in the decline in the searching efficiency per female for both *C. plutellae* and *D. mollipla*, but the killing power increased (Table 3.6). However, no significant differences were observed between the searching efficiency per female and parasitoid density (F = 1.22 NS, d.f. = 5, 24, P = 0.331) for *C. plutellae* (Table 3.6); the mutual interference (*m*) value for this parasitoid was 0.145. Likewise, no significant differences were observed between searching and parasitoid density (F = 1.31 NS, d.f. = 5, 24, P = 0.294) for *D. mollipla* (Table 3.6), even though the mutual interference value was higher at 0.343. No significant differences (*C. plutellae*: F = 1.22 NS, d.f. = 5, 24, P = 0.331; *D. mollipla*: F = 1.30 NS, d.f. = 5, 24, P = 0.298) were observed in killing power for both parasitoid species as the female density is increased (Table 3.6).

Table 3.4. Effect of parasitism on mortality of *Plutella xylostella* larvae at different host and parasitoid densities, and the effect of parasitoid density on the sex ratio (proportion of females) of progeny.

Control			Experiments							
Host density	Mortality		Host density	Mortality (one parasitoid) (%)		Parasitoid density	Mortality (100 hosts) (%)		Parasitoid density (100 hosts)	% females	
	Larvae	Pupae		*C. plutellae*	*D. mollipla*		*C. plutellae*	*D. mollipla*		*C. plutellae*	*D. mollipla*
20	3.2	3.4	20	7.8	9.2	1	61.8	65	1	17.64	35.29
40	7.8	14.4	40	12.4	18.4	2	62.6	47.8	2	14.45	28.39
60	3.8	17.2	60	21.4	35.4	4	66.4	52.8	4	23.81	18.75
80	12.4	23	80	26.4	50.8	6	53.4	56.4	6	37.96	24.39
100	13.4	25.8	100	61.8	65	8	68.8	66.4	8	39.62	27.01
						10	70.2	85.8	10	43.14	11.11

Table 3.5 The effect of different *Plutella xylostella* larval densities on the searching efficiency and killing power of *Cotesia plutellae* and *D. mollipla.* Values in the same column followed by the same letter are not significantly different.

	Area of discovery		Killing power	
Host density	*D. mollipla*	*C. plutellae*	*D. mollipla*	*C. plutellae*
20	2.67 ± 0.00a[1]	2.14 ± 0.47a[2]	1.16 ± 0.00a[3]	0.93 ± 0.2a[4]
40	1.00 ± 0.45b	0.51 ± 0.07b	0.44 ± 0.24b	0.21 ± 0.03b
60	0.64 ± 0.37b	0.24 ± 0.04b	0.28 ± 0.08b	0.10 ± 0.02b
80	0.69 ± 0.36b	0.17 ± 0.01b	0.30 ± 0.15b	0.05 ± 0.01b
100	0.23 ± 0.22b	0.12 ± 0.03b	0.10 ± 0.03b	0.07 ± 0.01b

LSD values ($P < 0.05$, Fisher's Protected LSD test): 1 = 0.898; 2 = 0.6294; 3 = 0.39; 4 = 0.2731

Table 3.6 The effect of different female parasitoid densities on the searching efficiency and killing power of *Cotesia plutellae* and *D. mollipla.* Values in the same column followed by the same letter are not significantly different.

	Area of discovery		Killing power	
Parasitoid density	*D. mollipla*	*C. plutellae*	*D. mollipla*	*C. plutellae*
1	0.23 ± 0.08a[1]	0.1692 ± 0.03a[2]	0.11 ± 0.03a[3]	0.07 ± 0.01a[4]
2	0.16 ± 0.02a	0.1572 ± 0.06a	0.07 ± 0.01a	0.07 ± 0.03a
4	0.47 ± 0.17a	0.0986 ± 0.02a	0.21 ± 0.08a	0.04 ± 0.01a
6	0.42 ± 0.13a	0.2046 ±0.14 a	0.18 ± 0.06a	0.09 ± 0.06a
8	0.39 ± 0.1a	0.1412 ± 0.02a	0.17 ± 0.04a	0.06 ± 0.01a
10	0.39 ± 0.04a	0.3122 ± 0.06a	0.17 ± 0.02a	0.14 ± 0.02a

LSD values ($P < 0.05$, Fisher's Protected LSD test): 1 = 0.3008; 2 = 0.1938; 3 = 0.1318; 4 = 0.0844

3.6 Discussion

Both *C. plutellae* and *D. mollipla* showed a greater preference for second and third instar hosts than for fourth instars, an indication of the possible competition between the two parasitoid species. However, *D. mollipla* attacked a higher proportion of fourth instars than *C. plutellae*. The ability of *D. mollipla* to parasitize and develop successfully in all three instars of *P. xylostella* gives it a wider niche than *C. plutellae*. Although a parasitoid can develop successfully in different larval instars of the same host, the cost of parasitism may vary between different host instars (Godfray 1994). Hosts (particularly lepidopteran larvae) are not necessarily helpless victims of their parasitoids (Godfray 1994; Jervis & Copland 1996). One of the most common responses of hosts to parasitoid attack is violent wriggling, and the movement may be sufficient to throw the parasitoid off the host, or to prevent it from laying an egg (Godfray 1994). A dramatic active host defense is when a host turns on its attacker and tries to kill it (Godfray 1994; Potting *et al.* 1999). Such an aggressive behaviour is commonly displayed by larger hosts (Jervis & Copland 1996; Potting *et al.* 1999; Seymour & Jones 2001).

Because hosts represent a finite resource, a female parasitoid's fitness is determined by the rate at which it finds hosts, the quality of the hosts it parasitises, and the number and sex of the eggs it lays. Host quality in turn influences three correlates of fitness in parasitoids: adult size, developmental time, and survival. Other studies have shown that host size affects other fitness-related characters, including longevity, fecundity and searching efficiency (Hemerik & Harvey 1999). Although it may be true in some parasitoid-host systems that by attacking bigger hosts the parasitoid increases the fitness of its offspring, an increase in host size or age does not necessarily mean an improvement in host quality (Pandey & Singh 1999). However, the rejection of apparently suitable hosts in koinobionts (i.e. parasitoid species that allow their hosts to grow and develop for some time after parasitism) suggests that host choice may not reflect current host quality but may be based on an assessment of future growth rates and resources available for the developing larvae (Rivero 2000). Because *C. plutellae*, a larval parasitoid, always emerges from fourth instars, it is beneficial for it to parasitize younger hosts in order for the developing parasitoid to complete development. Besides, larger hosts are better defended immunologically than smaller hosts (Godfray 1994). However, part of the reason for the low preference of fourth instars by *C. plutellae* is the ability of individuals in this instar to fight-off an approaching parasitoid. On those few occasions where a female parasitoid managed to attack a fourth instar *P. xylostella* larva, it did not spend as much time

spinning with it, a process that lasts a couple of seconds when it parasites second or third instars. This can be assumed to be the reason why parasitism of fourth instars yielded mainly male offspring in *C. plutellae*. Although *D. mollipla* laid mainly female eggs on second instar hosts compared to third or fourth instars in the *no-choice test*, it laid mostly female eggs on fourth instar hosts compared to second or third instars in the *choice test*. The reason for *D. mollipla* to exhibit female-biasness towards fourth instars when all host stages were available could be its ability to cope and parasitise the aggressive fourth instars. Being a larval-pupal parasitoid, *D. mollipla* is better able to utilise tissues of an advanced larval stage than a larval parasitoid. Because *D. mollipla* completes feeding just after a *P. xylostella* larva has pupated, it makes sense for it to lay female eggs on larger instars, as these have grown considerably in size and quality. Hence, the rate of development in fourth instars was significantly shorter than in second and third instars. The development rate of *C. plutellae* on second and third instars was significantly longer than development on fourth instars. Mackauer & Sequeira (1993, in Shi *et al.* 2002) proposed that parasitoids attacking low quality (i.e. small) hosts are predicted to exhibit a lag phase in development that allows the host to increase in size, whereas parasitoids attacking high quality (i.e. bigger) hosts are predicted to develop at a constant rate. Thus parasitoid developmental time varies with host quality at oviposition to maximise wasp biomass per unit of host resources. It is important to note that a more advanced host stage (fourth instar in this case) also means that the host internal organs are about to, or have started to, change in preparation of the next developmental stage. Therefore, for a larval parasitoid the more advanced the development of its host is, the more limited is the amount of the host resources.

Diadegma mollipla was almost twice fecund as *C. plutellae*. Studies on reproduction have tended to assume that the fecundity schedule is shaped mainly by survivorship (Dixon & Agarwala 2002). However, in the field as compared to the laboratory, very few individuals live long enough to reproduce in the second half of their lives than in the first. Thus natural selection would be expected to favour individuals that invest more in early reproduction even if it has an adverse effect on their potential longevity (Dixon & Agarwala 2002). As is assumed that the number of cocoons produced by the two parasitoid species per day represents egg production, then *C. plutellae* and *D. mollipla* produced about the same number of offspring in the first two days after eclosion. Using data of several parasitoid species that have been used in classical biological control programs against a variety of insect pests, Lane *et al.* (1999) tested the hypothesis that a parasitoid species with higher fecundity is a better

biological control agent because of its ability to kill a greater number of hosts over the course of its lifetime. The only relationship between fecundity and biological control success was detected in parasitoids of homoptera, and not for lepidoptera, perhaps because the former are sedentary external plant feeders and have less of a refuge from parasitism than lepidopteran larvae, which are either more mobile as external feeders or are protected as internal feeders within plant tissue (Lane *et al.* 1999). In addition to reproductive potential, host-searching efficiency would be of particular importance in parasitoids attacking mobile host stages. For instance, Drost *et al.* (2000) argued that a parasitoid with high fertility but poor host-searching efficiency may never encounter hosts in which to deposit its eggs.

Foraging success, or the ability of parasitoids to find and parasitize hosts, is of great importance in understanding population dynamics and interactions between species. Encounters with parasitized hosts lead to a greater incidence of leaving the patch in some parasitoid species (Outreman *et al.* 2001). Therefore, an ability to differentiate between parasitised and unparasitised hosts may in fact determine the extent of patch exploitation. Suppose there are two parasitoids, species A and B: A leaves the patch once it has come into contact with two parasitized hosts, whereas parasitoids B, on the other hand, searches for more hosts. The result would be that in each patch, parasitoid B would attack more hosts than parasitoid A, and that may lead B to occur in high densities in patches than A. According to Tenhumberg *et al.* (2001), a long patch residence time will reduce the percentage of hosts escaping parasitism, but may also result in superparasitism or time wasting by inspecting and rejecting already parasitized hosts. *Diadegma mollipla* females have been observed to show aggressive behaviour towards conspecific females in rearing cages, as a result there was an increased tendency to leave cabbage leaves with hosts, and spend time on the walls of the cages. *Cotesia plutellae* females were seldom seen on the walls of the cages. Even on encountering each other during searching, the females simply ignored each other. This difference in behaviours might explain the higher mutual interference value exhibited by *D. mollipla* compared to that of *C. plutellae.*

Besides superparasitism, high parasitoid densities are known to result in male-biased sex ratios in progeny. The reason for the male-biased sex ratios in progeny at high female densities can be explained through Local Mate Competition (LMC) theory. The LMC models predict what offspring sex ratio a mother should produce given the number of other mothers present (Hamilton, 1967 & 1979 in King 1993). If encounters between searching females are

more frequent than encounters with males, this situation results in sons being more valuable than daughters and thus there is a strong selection on the mothers to bias their progeny sex ratios towards males (King 1993). In this study, however, the increase in parasitoid density appeared to influence progeny sex ratio towards males only in *D. mollipla*. In contrary, an increase in parasitoid density resulted in female-biasedness in *C. plutellae.*

CHAPTER 4:

Effects of temperature on *Plutella xylostella* parasitism by *Cotesia plutellae* and *Diadegma mollipla*, and on their rates of development and emergence

4.1 Introduction

Insects are generally ectothermic; that is, they derive majority of their body heat from the environment (Chiang 1985). Temperature directly affects the dynamics of insect populations by affecting birth, death and growth rates of individuals. Thus temperature is an important driving force of insect population growth (Bommarco 2001). According to Sharpe & De Michele (1977) the rate of metabolism (and thus growth) of an ectothermic organism is driven by a rate-determining enzyme or enzyme complex, which has three basic reversible states: inactive at cold temperature, active at median temperature and inactive at hot temperature. For each insect species, there are temperature limits below and above which no development occurs called threshold temperature and thermal limit, respectively. Within this temperature range developmental rate increases with temperature and levels off at the optimum. Thus the relationship between temperature and development is curvilinear at low and high temperatures and practically linear at intermediate temperatures (Campbell *et al.* 1974; Chiang 1985; Dent 1991; Jervis & Copland 1996; Roy *et al*. 2002). The rate of development of an insect is determined by the rate of accumulation of heat units above a threshold temperature. The sum of accumulated heat units is normally referred to as a thermal constant, and is the cumulative product of total developmental time (in days or hours) multiplied by the temperature above the developmental threshold. It is expressed in degree-days or degree-hours (Dent 1991; Urbaneja *et al*. 2002). By monitoring daily temperatures and relating these to species-specific temperature-dependent growth curves, it is possible to predict a species' time of peak activity or its first seasonal occurrence (Romoser & Stoffolano 1994).

Generally, parasitoids have a narrower thermal tolerance than their hosts, therefore the wider the climatic range in which a pest occurs, the greater the number of parasitoid species that would be needed for its control (Romoser & Stoffolano 1994). Strains of the same parasitoid species from different ecological zones can tolerate different climatic conditions (Messenger & Bosch 1971). There are many examples of classical biological control programmes whose success depended on introductions of parasitoids from different ecological zones (Bosch *et al*. 1992). Introductions of *Plutella xylostella* parasitoids from Europe have, to a certain extent, reduced the pest status of *P. xylostella* in many countries or regions (Lim 1986; Waterhouse & Norris 1987; Ooi 1992; Talekar & Shelton 1993). Introductions of these parasitoids into countries with hot climates, such as the lowlands of Southeast Asia, have failed to provide effective control against *P. xylostella* (Talekar 1997; Verkerk & Wright 1997). Presently, the greatest challenge for biological control programmes against *P. xylostella* in this and similar

regions, such as the temperate hot and dry East Africa, is the absence of effective heat-tolerant parasitoids (N.S. Talekar, AVRDC, Taiwan, personal communication; B. Löhr, ICIPE, Kenya, personal communication). In Southeast Asia, for instance, the only parasitoid known to survive the hot and humid climate of the lowlands is *Cotesia plutellae*. However, it is in this region that frequent *P. xylostella* outbreaks occur and additional parasitoids are needed to supplement *C. plutellae* (Verkerk & Wright 1997). Biological control of *P. xylostella* in this and similar areas can benefit from introductions of heat tolerant and more efficient strains of *C. plutellae* (Roush 1997).

South Africa is predominantly a warm and dry country, exhibiting climatic conditions that normally lead to *P. xylostella* outbreaks in many countries (Talekar & Shelton 1993). However, the pest status of *P. xylostella* is low in South Africa compared to other countries of similar climate (Kfir 1997). The low pest status is due to the presence of a rich and effective guild of primary parasitoids attacking various developmental stages of *P. xylostella* (Kfir 1997). *Cotesia plutellae* is the most efficient parasitoid of *P. xylostella* in South Africa, and normally accounts for more than 80% of total parasitism (Kfir 1997; Waladde *et al*. 2001; Smith 2002; Mosiane *et al.* 2003). This is interesting considering that the most abundant parasitoid in East African countries (Kenya, Uganda, Tanzania and Ethiopia), which have a somewhat similar climate to South Africa, is *Diadegma mollipla* with *C. plutellae* playing an insignificant role (B. Löhr, personal communication). In South Africa, *D. mollipla* plays a minor role in the biological control of *P. xylostella*, and is active mainly in spring (September-November), whereas *C. plutellae* is the most abundant parasitoid throughout the year (Kfir 1997; Waladde *et al*. 2001; Mosiane *et al.* 2003). Total parasitism of *P. xylostella* rarely exceeds 15% in East Africa, whereas it is very high (normally >90%) in South Africa (Kfir 2003). Based on the high levels of parasitism of *P. xylostella* by *C. plutellae* in South Africa compared to other countries, there are plans to introduce *C. plutellae* from South Africa into East Africa, as part of a current biological control-based IPM programme for *P. xylostella* in Eastern and Southern Africa.

This study addressed the effects of temperature on parasitism of *P. xylostella* by *C. plutellae* and *D. mollipla*, and on their rates of development and emergence. Because *P. xylostella* is a major pest in tropical and subtropical regions, trials were conducted under temperatures that represent these climates where daily temperatures normally fluctuate between 21-33°C (Talekar 1997; Verkerk & Wright 1997).

4.2 Materials and methods

The studies were conducted in Labcon® LTGC 40 incubators set at constant temperatures of 21, 24, 27, 30 and 33 ± 0.5°C (mean ± s.d.). For *D. mollipla*, however, an additional lower test temperature of 18°C was included. In all the incubators, the photoperiod was maintained at 16L: 8D, and relative humidity was maintained between 50 and 60%. Thirty third instar larvae of *P. xylostella* were placed on a cabbage leaf in a glass jar (500ml), thereby forming an experimental unit, and 35 replicates were used. The jars were closed with gauze held tightly with rubber bands. A mated but naive one-day-old female parasitoid was introduced into each jar and allowed to parasitise hosts for 24 hours. The parasitoids were provided with honey streaked thinly on the walls of the jars. After this period, the parasitoids were removed and the hosts were reared further on fresh cabbage leaves. The leaves were changed every second day until the larvae formed pupae or parasitoid cocoons formed. The number of *P. xylostella* larvae that died at each test temperature was recorded, and these were excluded from the calculations. Parasitoid cocoons were transferred into Petri dishes, and these were held in place with rubber bands The development of parasitoids was checked twice daily (at 08h00 and 16h00), from the third day after the start of the experiment, and the dates of pupation and adult parasitoid emergence were recorded. The time of pupal formation and parasitoid eclosion was determined as the midpoint between two consecutive observations between which the event occurred (Kfir *et al*. 1993; Roy *et al*. 2002). No differences were observed in the developmental time (egg-adult) between male and female *C. plutellae* at all the other test temperatures except for 24°C (Table 4.1). The duration of development (egg-adult) for male and female *D. mollipla* did not differ in all the temperatures tested (Table 4.2). Because the developmental time of male and female *C. plutellae* differed only at 24°C (Table 4.1), the data were combined for calculations. A minimum and maximum time to develop for each developmental stage (egg-pupa, pupa-adult) and generation time were determined and the analyses were based on the mean developmental time at each temperature.

Many studies have been conducted which empirically describe the relationship between the development of insects and temperature. This relationship is usually plotted as a developmental rate against a series of constant rearing temperatures. This relationship is curvilinear at extreme (low and high) temperatures but practically linear at intermediate temperatures (Campbell *et al.* 1974; Chiang 1985; Dent 1991; Jervis & Copland 1996; Roy *et al*. 2002). Due to the nonlinearity of the curve at extreme temperatures, the validity of using extrapolation of the linear portion of the curve to estimate the threshold for development (*c*)

has been criticised by several workers, and several other methods have been developed (Lamb 1992; Bernal & González 1993; Raworth 1994; Lactin *et al.* 1995; Lamb 1998; Legg *et al.* 1998; Ikemoto & Takai 2000). Most of these new methods appear to be more accurate, and they provide measures of error in their estimates. However, their use requires large numbers of test temperatures that include low, optimum and high temperatures. Therefore, their use is not always practical. This is especially true when a study, such as this one, deals only with temperatures in the middle and upper ranges. Besides, the increased accuracy that may be obtained from these new formulae may not be as essential for practical applications as it is for theoretical ones (Lamb 1992). Furthermore, the method of estimating developmental threshold from extrapolation of the linear portion of the curve has been used successfully for insect pests in the field (Dent 1991; Lamb 1992). Therefore, this method was used to estimate the threshold for development for *C. plutellae* and *D. mollipla.*

The equation of the equilateral hyperbola, based on the assumption that the product of development time and temperature above a certain threshold is a constant (thermal constant), describes the effects of temperature on the duration of development in insects (Bodenheimer, 1958 in Moore & Kfir 1996). The hyperbola equation [$ThC = t\ (T\text{-}c)$] for *C. plutellae* and *D. mollipla* was calculated as follows: rates of development for the temperatures (T) used were obtained as the reciprocals of the developmental periods (t) shown in Tables 4.3 and 4.4; the linear regression ($y = a + bx$) of the developmental rate on temperature was then calculated using the method of least squares; the parameters of the equilateral hyperbola (thermal constant = ThC and developmental threshold = c) were then obtained from the identities $ThC = 1/b$; $c = -a/b$ (Moore & Kfir 1996).

4.3 Data analysis

To determine whether there were any differences in parasitism of *P. xylostella*, duration of development and emergence of *C. plutellae* and *D. mollipla* at different temperatures, the data were subjected to Analyses of Variance followed by multiple comparison Fisher's Protected Least Significant Difference test. Before analysis, data were subjected to a Bartlett test for homogeneity and Watson test for normality. The data were analysed using the statistical program Genstat 5 (Genstat for Windows 2000).

4.4 Results

At each temperature tested, using 95% confidence intervals, the developmental time (egg-adult) for male and female *C. plutellae* was not significantly different (21°C: t = 0.73, d.f. = 277, p = 0.463; 27°C: t = -0.03, d.f. = 67, p = 0.972; 30°C: t = -1.18, d.f. = 95, p = 0.239; 33°C: test inconclusive due to a small (<5) sample size), except for 24°C (t = -5.77, d.f. = 275, $P < 0.001$). At all temperatures tested, using 95% confidence intervals, the duration of development (egg-adult) for male and female *D. mollipla* were not significantly different (18°C: t = -0.98, d.f. = 202, p = 0.327; 21°C: t = -0.45, d.f. = 101, p = 0.654; 24°C: -1.38, d.f. = 46, p = 0.174; 27°C: t = 0.19, d.f. = 55, p = 0.854; 30°C: t = -0.2, d.f. = 12, p = 0.841).

The developmental time for *C. plutellae* decreased significantly ($F = 3094.56$, d.f. = 4, 130, $P < 0.01$) with an increase in temperature (Table 4.3). The duration of development for *D. mollipla* was also significantly shorter ($F = 4809.45$, d.f. = 4, 127, $P < 0.01$) as the temperature increased (Table 4.4). *Cotesia plutellae* completed development at all test temperatures (Table 4.3) whereas *D. mollipla* completed development at temperatures up to 30°C (Table 4.4). As no development occurred above 30°C, it can be concluded that the upper threshold for development for *D. mollipla* is around 31°C. The linear regression was calculated as $Y = -0.037 + 0.0046X$ (s.e. for intercept = 0.006; s.e. for slope = 0.00025; $r^2 = 0.72$; r = 0.85; $p \leq 0.001$) for *C. plutellae*, and for *D. mollipla* it was calculated as $Y = -0.043 + 0.0042X$ (s.e. for intercept = 0.002; s.e. for slope = 0.00009; $r^2 = 0.94$; r = 0.97; $p \leq 0.001$). *Cotesia plutellae* required 217.39 degree-days (*ThC*) above 8.14°C (*c*) (Fig. 4.1A) to develop from egg to adult, whereas *D. mollipla* required 238.1 degree-days above 10.23°C (Fig. 4.1B).

At all temperatures tested, the duration of development (egg-adult) for *C. plutellae* was shorter than that of *D. mollipla* (Tables 4.3 and 4.4). Because both parasitoid species prefer to attack the same host stages (i.e. second and third instars), the shorter generation time for *C. plutellae* gives it a competitive advantage over *D. mollipla*. Parasitism levels of *P. xylostella* larvae were generally higher for *D. mollipla* than *C. plutellae* at all test temperatures (Tables 4.3 and 4.4). *Plutella xylostella* parasitism by *D. mollipla* was highest at 24°C, and there was a decline in the proportion of hosts parasitised both at higher and lower temperatures (Table 4.4). For *C. plutellae*, parasitism was highest at 21°C and decreased with increasing temperature (Table 4.3). Emergence of *D. mollipla* (Table 4.4) was rather low compared to *C. plutellae* (Table 4.3), and for both parasitoid species the eclosion of adult parasitoids

decreased with increasing temperature. An increase in temperature did not appear to affect the sex ratio (proportion of males) in the progeny (Tables 4.3 and 4.4).

Studies have shown that the survival of *P. xylostella* larvae is high at lower temperatures (<20°C) than at higher temperatures (>25°C) (Wakisaka 1992; Liu *et al.* 2002; Smith 2002). It is most probable therefore that the recorded parasitism rates of *P. xylostella* at higher temperatures may have been under estimated due to the lower survival of the hosts.

4.5 Discussion

The study demonstrated that *C. plutellae* was able to develop in a wide range of temperatures (8.14-33°C) compared to *D. mollipla* (10.23-30°C). Because of wider thermal tolerance of *C. plutellae* compared to *D. mollipla*, and the fact that the former had a shorter generation time than the latter at all temperatures tested, it can be argued that both these factors contribute to the dominance of *C. plutellae* over *D. mollipla* in the field in South Africa.

The biology of *D. mollipla* has never been studied on *P. xylostella*; therefore there is no information on its thermal requirements in association with this host. Broodryk (1971) studied temperature requirements of *D. mollipla* on potato tuber moth, *Phthorimaea operculella* (Zeller). However due to possible differences in host suitability it would be erroneous to compare findings in this study with that of Broodryk's. The biology of *C. plutellae* has been studied extensively, including its temperature requirements, particularly in Southeast Asia. Lim (1982) reported that the developmental threshold for the Malaysian strain of *C. plutellae* is 13.8°C, which is much higher than the 8.14°C estimated for the South African strain. In addition, Talekar & Yang (1991) reported that a temperature range of 20-35°C is more suitable for the Taiwanese strain of *C. plutellae*. Considering both reports (Lim 1982; Talekar & Yang 1991), it is not a coincidence that the strain of *C. plutellae* in Southeast Asia is more dominant in the hot and humid lowlands. The low threshold for development (8.14°C) in the South African strain of *C. plutellae* and its ability to complete development at temperatures as high as 33°C means this parasitoid is adaptable to varied climatic conditions. As an example, the South African strain of *C. plutellae* was introduced into St. Helena (a tropical island in the South Atlantic Ocean), together with *Diadromus collaris* (Gravenhorst) (a pupal parasitoid), where they, together with an indigenous strain of *D. mollipla*, caused total biological control of *P. xylostella* in the first year of their release (Kfir & Thomas 2001). *Cotesia plutellae* is now widespread throughout the island (Kfir & Thomas 2001).

This study revealed that the South Africa strain of *C. plutellae* has a wider thermal tolerance compared to the Southeast Asian strain, and can be a suitable biological control agent for release against *P. xylostella* in East Africa. Thermal tolerance studies, however, reveal only whether or not a given parasitoid can be a suitable candidate for release under given climatic conditions- on their own they are not sufficient to indicate the level of competency of an agent. In this view, it is essential to consider results of this study with those of other biological characteristics such as fecundity, searching efficiency, host stage preference, proportion of females in the progeny, etc.

Table 4.1 Duration of development of male and female *Cotesia plutellae* at five constant temperatures. Values in the same column followed by an asterisk (*) are significantly different.

	Temperature (°C)				
	21	24	27	30	33
Male	20.63 ± 0.05	11.47 ± 0.08	10.71 ± 0.13	10.37 ± 0.07	9 ± 0.00
Female	20.58 ± 0.06	12.10 ± 0.07*	10.71 ± 0.15	10.50 ± 0.08	9 ± 0.00

Table 4.2 Duration of development of male and female *Diadegma mollipla* at five constant temperatures. Values in the same column followed by an asterisk (*) are significantly different.

	Temperature (°C)				
	18	21	24	27	30
Male	29.78 ± 0.14	24.37 ± 0.12	15.68 ± 0.07	13.11 ± 0.04	12.42 ± 0.15
Female	30.03 ± 0.21	24.56 ± 0.34	15.95 ± 0.18	13.1 ± 0.07	12.5 ± 0.5

Table 4.3. Effects of temperature on *Plutella xylostella* parasitism by *Cotesia plutellae*, and on its rates of development and emergence and sex ratio at five constant temperatures. Values in the same column followed by the same letter are not significantly different.

Temperature (°C)	n	Duration of development (days) (Mean ± S.E.)			% Parasitism (Mean ± S.E.)	% Emergence (Mean ± S.E.)	Sex ratio (male: female)
		egg-pupa	pupa-adult	egg-adult			
21	746	13.93 ± 0.14a[1]	6.61 ± 0.15a[2]	20.54 ± 0.06a[3]	63.8 ± 3.32a[4]	67.9 ± 2.91a[5]	1.79: 1
24	610	8.04 ± 0.1b	3.94 ± 0.09b	11.98 ± 0.05b	65.9 ± 3.79a	71 ± 3.63a	1.47: 1
27	240	6.93 ± 0.13c	3.89 ± 0.09b	10.60 ± 0.11c	50.9 ± 5.64b	66.1 ± 5.35a	1.46: 1
30	330	6.7 ± 0.08cd	4.03 ± 0.11b	10.96 ± 0.1d	48.7 ± 4.41b	71.1 ± 5.05a	1.22: 1
33	52	6.51d	2.52c	9e	29 ± 3.36c	40.2 ± 8.73b	1.5: 1

LSD values ($P < 0.05$, Fisher's Protected LSD test): 1 = 0.3094; 2 = 0.3175; 3 = 0.2291; 4 = 11.92; 5 = 13.23.

Table 4.4. Effects of temperature on *Plutella xylostella* parasitism by *Diadegma mollipla*, and on its rates of development and emergence and sex ratio at five constant temperatures. Values in the same column followed by the same letter are not significantly different.

Temperature (°C)	n	Duration of development (days) (Mean ± S.E.)			% Parasitism (Mean ± S.E.)	% Emergence (Mean ± S.E.)	Sex ratio (male: female)
		egg-pupa	pupa-adult	egg-adult			
18	450	11.81 ± 0.12a[1]	18.72 ± 0.17a[2]	30.53 ± 0.1a[3]	67.3 ± 4.21bc[4]	67.2 ± 4.19a[5]	2.4: 1
21	363	7.86 ± 0.12b	16.34 ± 0.12b	24.2 ± 0.15b	71.6 ± 5.1bc	37.1 ± 4.24b	10.44: 1
24	370	6.18 ± 0.07c	9.59 ± 0.08c	15.73 ± 0.05c	84.9 ± 3.92a	59.7 ± 3.58a	4: 1
27	207	4.54 ± 0.07d	9.13 ± 0.13d	13.64 ± 0.11d	79.8 ± 4.69ab	38.7 ± 5.89b	2: 1
30	170	4.64 ± 0.06d	8.1 ± 0.12e	12.74 ± 0.11e	65 ± 4.86c	16.9 ± 5.12c	6: 1
33	no development						

LSD values ($P < 0.05$, Fisher's Protected LSD test): 1 = 0.2619; 2 = 0.1293; 3 = 0.3112; 4 = 12.79; 5 = 13.10.

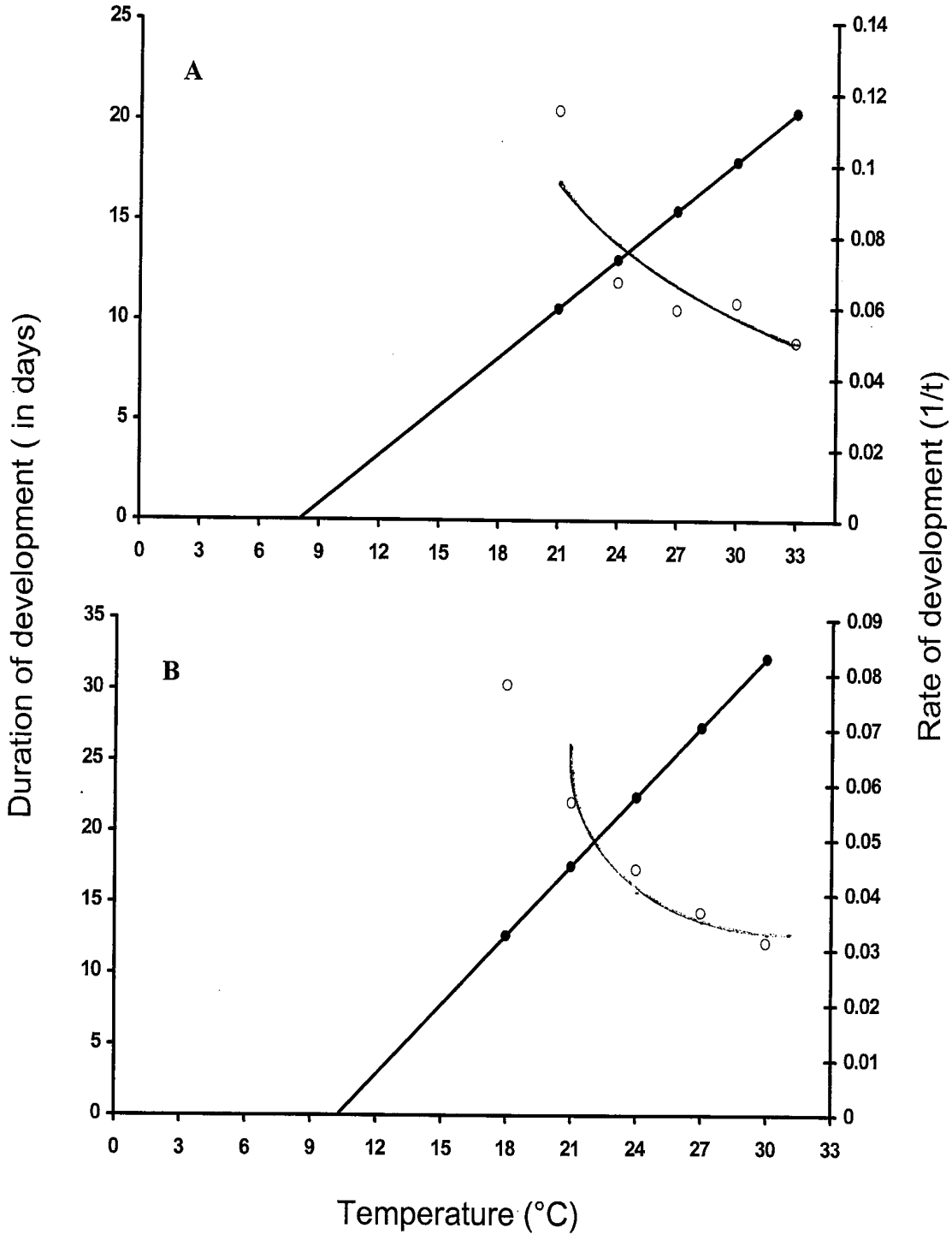

Fig. 4.1 Effect of temperature on the duration and rate of development of *Cotesia plutellae* (**A**) and *Diadegma mollipla* (**B**). Open circles represent duration of development; solid circles represent rate of development

CHAPTER 5:

The role of parasitoids in regulating diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), populations on unsprayed cabbage

5.1 Introduction

Cabbage, *Brassica oleracea* L. variety *capitata*, is an important vegetable crop in South Africa, particularly in rural areas where it is grown for subsistence (Charleston 1998). Commercially, *ca.* 9 000 hectares are planted annually resulting in a harvest of about 450 000 tons per annum (Kfir 1997). Successful production of cabbage, and other cruciferous crops, is threatened by a variety of insect pests of which diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered the most important (Ullyett 1947; Annecke & Moran 1982; Mosiane 2001). *Plutella xylostella* is cosmopolitan in distribution, and is a major pest of cruciferous crops in many other countries throughout the world (Talekar & Shelton 1993). The earliest published record of *P. xylostella* in South Africa was made in the early 1900s by Gunn (1917), who also studied its biology. Later, in an attempt to develop a management strategy for this pest, Ullyett (1947) studied its natural mortality factors, and cited parasitoids as the most important mortality factor acting in a density-dependent manner. However, given the easier option of using insecticides, no attempt was made to exploit the use of parasitoids against *P. xylostella*.

For decades, *P. xylostella* was not a problem in South Africa, as insecticides seemed capable of providing effective control against it. In the early 1990s, however, reports of *P. xylostella* outbreaks and subsequent yield losses became common (Dennill & Pretorius 1995). It was subsequently established that the extensive and regular use of insecticides has led local populations of *P. xylostella* to develop resistance to the commonly used chemicals (Sereda *et al.* 1997). In some areas, insecticide resistance is so serious that farmers, in an attempt to overcome it, spray their crops twice a week while others use high doses and/or cocktails of insecticides (Dennill & Pretorius 1995; Sereda *et al.* 1997). Because most of the chemicals used by farmers are broad-spectrum insecticides (Sereda *et al.* 1997), it is possible that the intensive use of these chemicals has not only led to the development of resistance in *P. xylostella* but also to the exclusion of natural enemies in these areas. Therefore, *P. xylostella* outbreaks are both the result of the failure of insecticides to provide effective control against it and the absence of its natural enemies. For instance, Dennill & Pretorius (1995) recorded only one parasitoid of *P. xylostella* in an area (Pretoria region) where cabbages were protected by intensive use of insecticides whereas Kfir (1997), in the same region, reared a complex of 21 species of parasitoids from *P. xylostella* where no insecticides were used. Kfir (2003) demonstrated by using the insecticidal check method that over-reliance on insecticides is detrimental to parasitoids, as *P. xylostella* occurred in much higher densities in sprayed

cabbage fields compared to cabbage grown under insecticide-free conditions. The negative impact of insecticides on parasitoids and consequently on the control of *P. xylostella* is well documented (Lim 1986; Ooi 1992; Idris & Grafius 1993; Syed *et al.* 1997; Hill & Foster 2000).

As a follow up of Kfir's (1997) work on parasitoids of *P. xylostella* in the North West Province, South Africa, the study reported here was undertaken to determine (i) the flight patterns of male moths, (ii) seasonal fluctuations in *P. xylostella* infestations on unsprayed cabbage plots and the relative abundance of each of the developmental stages in relation to the age of the crop and, (iii) composition, seasonality and relative abundance of its parasitoids. In addition, the role of abiotic factors, particularly temperature and rainfall, on population dynamics of *P. xylostella* was assessed.

5.2 Materials and methods

Flight patterns of *P. xylostella* male moths

The flight activity of *P. xylostella* male moths was monitored with synthetic sex pheromone traps in unsprayed cabbage plots at Brits-Agricultural Research Station (25°25'33"S, 27°76'67"E, altitude 1102m) in the North West Province, South Africa. Three delta-shaped traps (Biotrap®), (26 X 9.5 X 13cm), were deployed in each cabbage plot (47 X 20m). The traps were mounted on steel posts erected around the cabbage plot at approximately 1m above the ground. Waxy paperboards were cut to fit the sliding bottom panels of the traps, thus covering the trap floors. The floors were then sprayed with a sticky polybutene adhesive. The lure, a small cylindrical-shaped rubber impregnated with a formulation of *P. xylostella* sex pheromone (supplied by Agrisense-BCS Limited, UK), was placed at the center of the sticky floor in each trap. The sticky floors were replaced at weekly intervals after counting the number of moths caught in each trap. The synthetic sex pheromones were replaced every five weeks.

Seasonal abundance of *P. xylostella* larvae and pupae, and of its parasitoids

Cabbage seedlings were transplanted in three consecutive times a year for two years (February 2000-January 2002) in plots of about 47 X 20m (0.5m between plants and 1m row spacing). Standard cultivation practices that include fertilising, weeding and irrigation were followed. Natural infestation of pests was allowed to take place without any application of insecticides. Scouting for *P. xylostella* larvae and pupae commenced two weeks after

transplanting the new cabbage seedlings in the field. At weekly intervals, 30 plants selected randomly were inspected and the number of *P. xylostella* larvae, pupae and parasitoid cocoons found in each plant recorded. In order to determine parasitism, samples of third and fourth instar larvae, pupae and parasitoid cocoons were collected. The samples were taken to the laboratory where they were maintained at 25 ± 1°C (mean ± s.d.), 65 ± 5% RH and 16: 8 (L: D) photoperiod. The larvae were provided with sections of fresh cabbage leaves and held singly in Petri dishes. The leaves were replaced every second day until the larvae pupated or parasitoid cocoons formed. Samples of parasitoid cocoons and *P. xylostella* pupae were confined individually in ventilated glass vials (2.5 X 10cm). Emerging parasitoids were identified and their incidence calculated. The number of moths that emerged from samples of larvae and pupae were also recorded. Larvae and pupae that died of unknown causes were excluded from calculations of parasitism.

Effects of temperature and rainfall on populations of *P. xylostella* and its parasitoids

Temperature and rainfall data were obtained (from the *South African Weather Service*, Pretoria) and their effects on population dynamics of *P. xylostella* and its parasitoids assessed.

5.3 Data analysis

To determine whether there is any relationship between the flight activity of *P. xylostella* and larval and pupal infestations on the crop, a correlation analysis was performed using Genstat 5 (Genstat for Windows 2000).

5.4 Results

Flight patterns of *P. xylostella* male moths

Pheromone trap catches indicated that *P. xylostella* moths were active throughout the year (Fig. 5.1). The flight activity of male moths followed a similar pattern each year throughout this study. Trap catches were low in summer (December-February) followed by slight increases in autumn (March-May) and winter (June-August), and peak moth activity in spring (September-November). Thereafter moth activity declined sharply towards the end of spring and in summer (Fig. 5.1).

Seasonal abundance of *P. xylostella* larvae and pupae

The flight activity of moths corresponded with larval infestations on the crops; the correlation between the two variables was significant ($P < 0.001$, d.f. = 44) in both years, but it was stronger ($r = 0.803$) for the 2000-2001 season compared to ($r = 0.425$) the 2001-2002 season. Infestations were low in summer (December-February), often fluctuating between zero and one larva per plant. Infestations increased to about four larvae per plant in autumn (March-May) and winter (June-August). Infestations increased further towards the end of winter reaching their peaks of 53 and 8 larvae per plant in 2000-2001 and 2001-2002, respectively, in spring, then infestation levels declined sharply and remained low throughout the summer period (Fig. 5.2). Even at low pest densities, a relatively high number of plants were infested (Fig. 5.2), an indication of a regular distribution of the pest. All the developmental stages of *P. xylostella* were present at all developmental stages of the crop (Fig. 5.3), an indication that *P. xylostella* occurred in overlapping generations.

Composition, seasonality and relative abundance of *P. xylostella* parasitoids

Eight species of hymenopteran parasitoids were reared from *P. xylostella*. The parasitoids (*C. plutellae*, *O. sokolowskii* and *A. halfordi*) were active throughout the year (Fig. 5.4), and were responsible for the high levels of parasitism that reached 100% on many occasions (Figs. 5.5 & 5.6). Hyperparasitoids were also active for most of the year (Fig. 5.4), however, hyperparasitism was generally low except for the spring and summer periods (Fig. 5.5).

Primary parasitoids: *Cotesia plutellae* (Kurdjumov) (Braconidae), a larval parasitoid, was active throughout the year and dominated the parasitoid complex (Fig. 5.4). *Oomyzus sokolowskii* (Kurdjumov) (Eulophidae), a larval-pupal parasitoid, was also active throughout the year and was the second most abundant parasitoid (Fig. 5.4). *Oomyzus sokolowskii* was the only gregarious parasitoid of *P. xylostella* recorded during this study. Although it was mainly recorded from samples of *P. xylostella* pupae (rarely from larvae), in the calculations of percentage parasitism it was considered to be a larval parasitoid. On a few occasions, *O. sokolowskii* also emerged from *C. plutellae* cocoons, but its activity as a primary parasitoid far exceeded its hyperparasitic activity. *Apanteles halfordi* (Ullyett) (Braconidae) [=*A. eriophyes* (Nixon) (Prinsloo 2002)], a larval parasitoid, was also recorded throughout the year, except in winter, but its activity was sporadic (Fig. 5.4). *Apanteles halfordi* is endemic to South Africa and has been recorded only from *P. xylostella* (Prinsloo 2002). Only a single specimen of *Diadegma mollipla* (Holmgren) (Ichneumonidae), a larval-pupal parasitoid, was

recorded, in spring, during this study. *Diadromus collaris* (Gravenhorst) (Ichneumonidae), the only pupal parasitoid recorded in this study, was active from May to November (Fig. 5.4).

Hyperparasitoids: except for *O. sokolowskii*, which is a facultative and gregarious hyperparasitoid, all the others are obligate and solitary hyperparasitoids. Although the obligate hyperparasitoids were active for most part of the year (Figs. 5.4), hyperparasitism was generally low except in spring (September-November) and summer (December-February) when it rose to 25% (Fig. 5.5). The most abundant hyperparasitoid was *Eurytoma* sp. (Eurytomidae), followed by *Mesochorus* sp. (Ichneumonidae) and *Pteromalus* sp. (Pteromalidae). *Eurytoma* sp. and *Pteromalus* sp. attacked *C. plutellae* cocoons and emerged from them. Kfir (1997) reported that *Pteromalus* sp. also attacks cocoons of *A. halfordi* and *D. mollipla*, and occasionally *D. collaris* inside *P. xylostella* pupae. *Mesochorus* sp. emerged from cocoons of field-collected *C. plutellae* and from cocoons that formed from field collected *P. xylostella* larvae. Clearly, *Mesochorus* sp. oviposits in a parasitised *P. xylostella* larva, and then the hyperparasitoid offspring completes development once the primary parasitoid has formed a cocoon. It then pupates inside the cocoon of its host and emerges from it.

5.5 Discussion

Although *P. xylostella* was active throughout the year with all its developmental stages present at any given time, fourth instar larvae and pupae were the most abundant developmental stages. Generally, an increase in advanced developmental stages indicates that infestations are declining, whereas a preponderance of younger larvae indicates an increase in the incidence of infestation. The observed higher incidence of fourth instar larvae over first or second instars, for instance, could simply be due to easy detection, and visibility, of the greener fourth instars compared to the paler first and second instars, particularly when larvae fall off the plant. In fact, Mosiane *et al.* (2003) demonstrated that a large number of younger instars is missed in visual scouting compared to when Berlese funnels are used. Despite *P. xylostella* being active throughout the year, cabbage infestations by *P. xylostella* larvae and pupae were generally low, except in spring, even though no insecticides were applied. Natural mortality factors of *P. xylostella* include biotic factors such as parasitoids, predators and diseases, and abiotic factors such as rainfall and high temperatures (Ullyett 1947; Wakisaka *et al.* 1992; Liu *et al.* 2000; Shirai 2000).

The increase in trap catches and infestation levels towards the end of winter and into spring appeared to correspond with increasing daily temperatures during this period (Fig. 5.7). However, a sharp decline in *P. xylostella* populations was observed towards the end of spring (September-November) and into summer (December-February). High temperatures (>30°C) have been shown, in laboratory experiments, to cause low larval survival and fecundity in *P. xylostella* (Shirai 2000). Although maximum temperatures in spring and summer periods exceeded 30°C on many occasions during this study (Fig. 5.7), under natural conditions temperatures fluctuate wildly and normally exceed 30°C only for short periods during the day. Liu *et al.* (2002) reported normal development and survival of *P. xylostella* larvae under alternating temperature regimes, even at temperatures as high as 38°C. It is therefore unlikely that the sharp decline in *P. xylostella* populations towards the end of spring, and the low *P. xylostella* populations in summer, are the result of high temperatures alone. Besides, population outbreaks of *P. xylostella* are known to occur mainly during hot and dry periods (Gunn 1917; Talekar & Shelton 1993). In contrast, *P. xylostella* is known to be very susceptible to high humidity (Wakisaka *et al.* 1992). Summer is the rainfall season in the study area, but a significant amount of rain fell in spring during this study (Fig. 5.8). Ullyett (1947) reported the presence of an entomophagous fungus, *Zoophthora radicans* (Brefeld) [=*Entomophthora sphaerosperma* Fres.], which causes catastrophic larval mortality after a prolonged (but not necessarily heavy) rainfall. Because most pathological conditions that occur among insects are induced by a favourable combination of temperature and humidity (Ullyett 1947), it can be assumed that the high temperatures and rainfall created an ideal environment for *Z. radicans* infection, which contributed to the decline in *P. xylostella* populations towards the end of spring and during summer periods. The combined effect of high temperatures and rainfall in reducing *P. xylostella* populations has also been reported by others (Wakisaka *et al.* 1992; Waladde *et al.* 2001). In addition to creating an ideal environment for *Z. radicans* infection, rainfall also reduces *P. xylostella* infestations by dislodging eggs from leaves and drowning young larvae (Ullyett 1947; Wakisaka *et al.* 1992; Talekar & Shelton 1993). For instance, the use of overhead irrigation, which simulates rainfall, has been shown to suppress *P. xylostella* infestations on cabbage (Talekar *et al.* 1986; Mchugh & Foster 1995). Because sprinkler irrigation was used at least once a week to water the cabbage plots, it is possible that some of the larvae were killed by this activity. Since the effect of rainfall or sprinkler irrigation is immediate, the pest population can recover and build up to high densities in their absence. An important question, therefore, is what kept *P. xylostella* populations low throughout the year, especially during dry periods?

Numerous parasitoids and predators attack all developmental stages of *P. xylostella* (Ullyett 1947; Talekar & Shelton 1993; Liu *et al* 2000). A number of predators that include birds, adult and/or immature stages of a variety of insect species are known to prey upon all developmental stages of *P. xylostella.* However, none of these predators are specific to *P. xylostella* and therefore their numbers cannot be correlated directly with the pest population (Gunn 1917; Ullyett 1947). Based on this information, no attempt was made to investigate the impact of predators on *P. xylostella* populations. Parasitoids, on the other hand, are more host-specific and can parasitise several hundred hosts in a density-dependent manner (Annecke & Moran 1982). For this reason, the impact of parasitoids on pest populations is considered to be more important than that of predators (Ullyett 1947; Annecke & Moran 1982; Talekar & Shelton 1993).

Parasitoids depend on their hosts for reproduction, so for parasitoids to attain densities needed to control a given pest there should be a continuous association between the parasitoid/s and pest populations (Dent 1991). In seasonal crops, the instability of the system produces a separation in space and time between the natural enemies and host populations (Dent 1991). In this study, the continuous association between *P. xylostella* and its parasitoids was maintained by transplanting new cabbage seedlings in the vicinity of the older cabbage plot. The older cabbage plants were kept, before being ploughed under, for at least three weeks after the new transplants. Parasitism of *P. xylostella* larvae and pupae has been reported to be higher towards the end of the season (Lim 1986; Talekar & Yang 1993), therefore the older plots may have served as reservoirs for the pest and its parasitoids that later moved to colonise the new cabbage plantings. This practice probably contributed to the very high levels of parasitism of *P. xylostella* larvae when they were still at low densities.

The high parasitism levels of *P. xylostella* at low densities mean that, for the pest to escape parasitism, there should be a factor that disturbs this association. Parasitism is strongly influenced by the interaction of parasitoids with other natural mortality factors acting on the same host (Ullyett 1947) or on the parasitoids themselves (Rosen 1976). The cold winter temperatures inhibited the activity of the parasitoids, as some were inactive during this period while those present occurred in low densities. Because of the low *P. xylostella* parasitism levels in winter the pest populations increased rapidly towards the end of winter and early spring. However, as soon as temperatures increased and became more favourable in spring, the parasitoids increased their activities to reduce the increasing *P. xylostella* populations.

Hyperparasitism was generally low and the only time that hyperparasitism was higher (5-25%) was during late spring and early summer, when the activity of parasitoids was very high (Fig. 5). However, the effect of hyperparasitism in regulating the primary parasitoids or the pest populations was minimal.

Because cabbage infestations by *P. xylostella* were generally low and levels of larval parasitism high, parasitoids could be considered the most important mortality factor of immature stages of *P. xylostella*. However, use of parasitoids alone cannot guarantee total control of *P. xylostella* or minimum damage to the crops throughout the year. The low parasitism of *P. xylostella* during winter and early spring might require other control measures or introductions of exotic parasitoids adapted to cooler conditions.

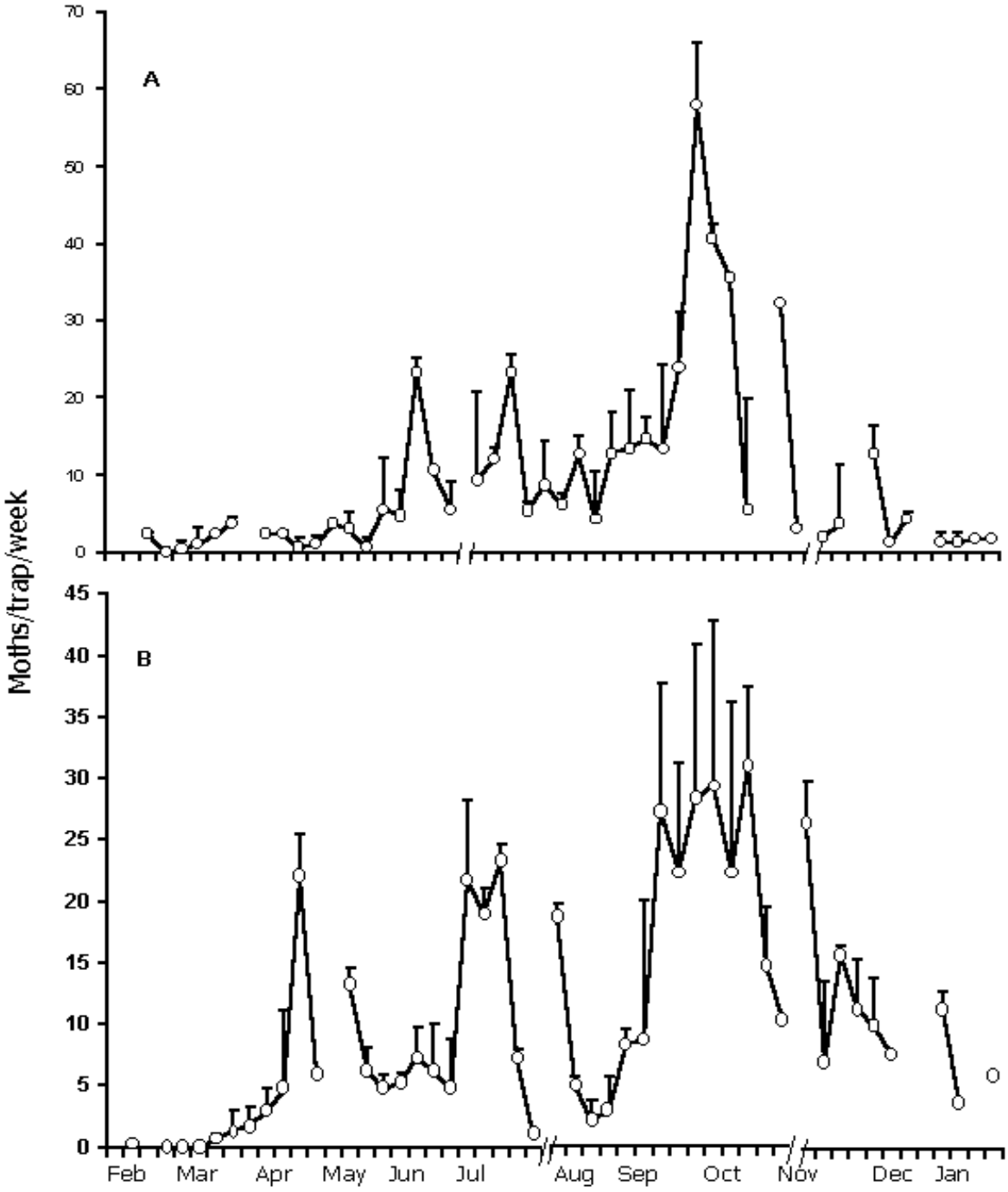

Fig. 5.1 . Flight activity of *Plutella xylostella* male moths as shown by synthetic sex-pheromone trap catches during February 2000-January 2001 (**A**) and February 2001-January 2002 (**B**) at Brits, South Africa. Bars represent standard errors (s.e.) when larger than symbol size. Diagonal lines on the x-axis represent transplanting of new cabbage seedlings.

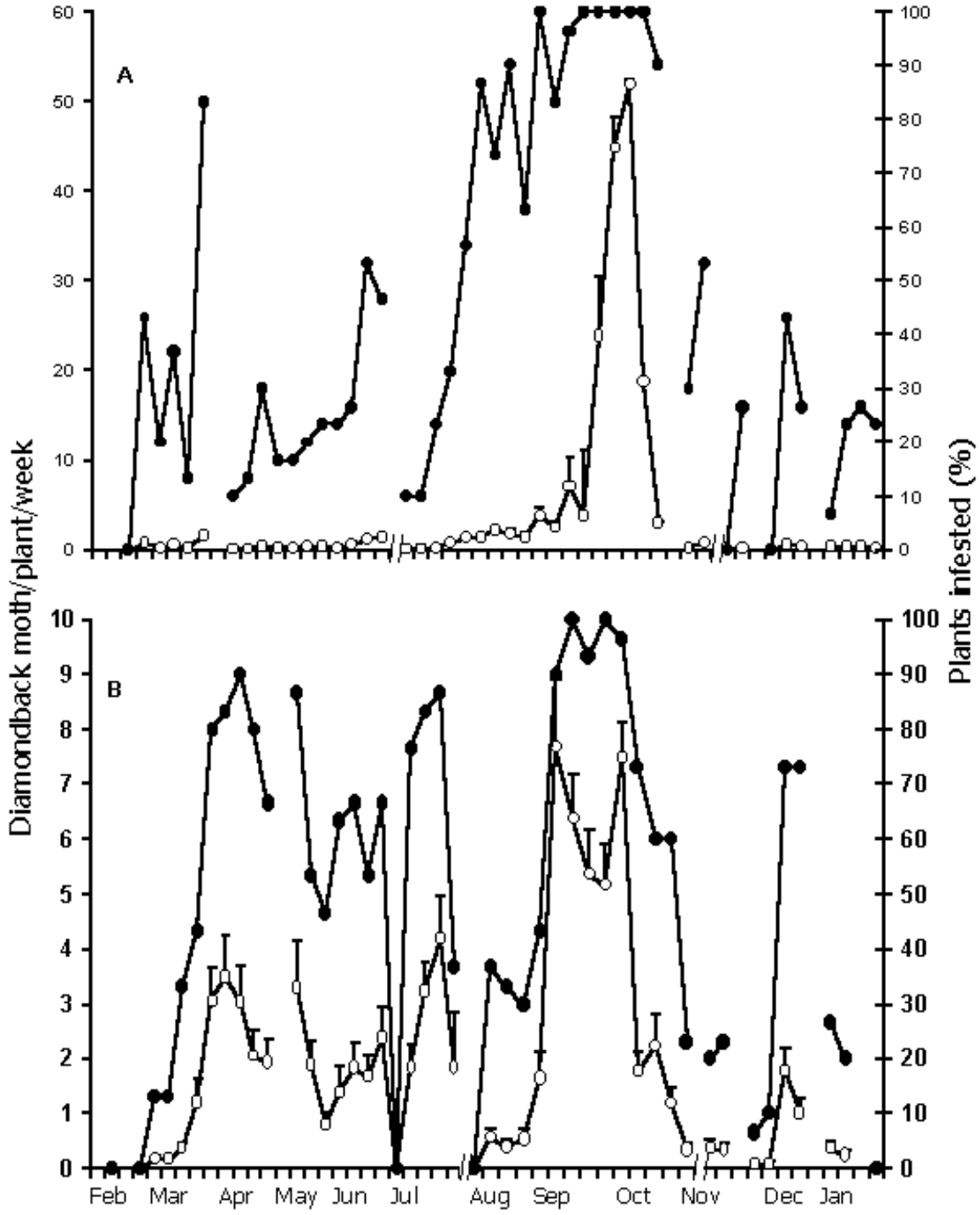

Fig. 5.2 Abundance of larvae and pupae of *Plutella xylostella* on unsprayed cabbage plots during February 2000-January 2001 (**A**) and February 2001-January 2002 (**B**) at Brits, South Africa. Open circles represent number of *P. xylostella* larvae and pupae per plant; solid circles represent proportion of plants infested. Bars represent standard errors (s.e.) when larger than symbol size. Diagonal lines on the x-axis represent transplanting of new cabbage seedlings.

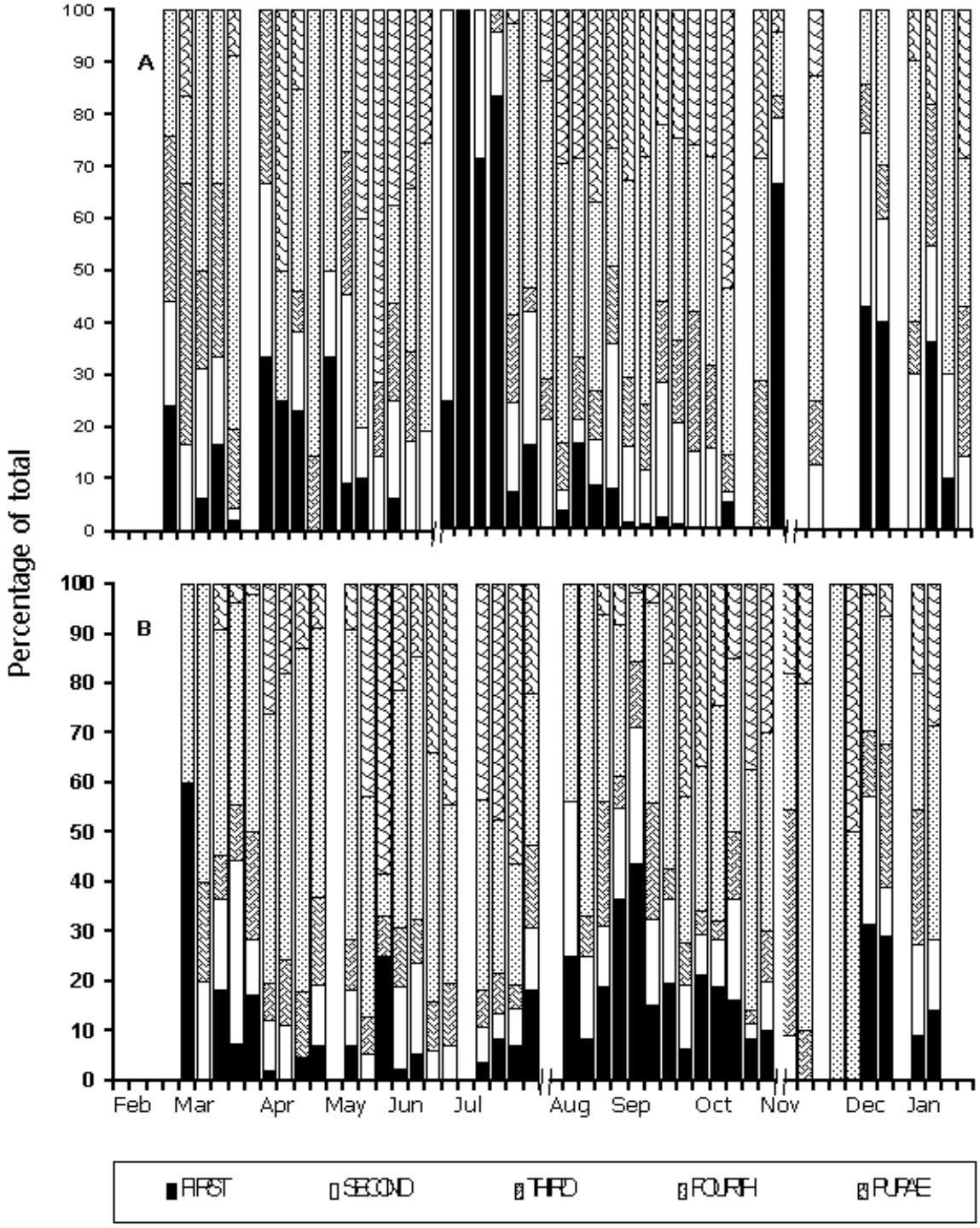

Fig. 5.3 . Relative abundance of *Plutella xylostella* larval instars and pupae on unsprayed cabbage plots during February 2000-January 2001 (**A**) and February 2001-January 2002 (**B**) at Brits, South Africa. Diagonal lines on the x-axis represent transplanting of new cabbage seedlings.

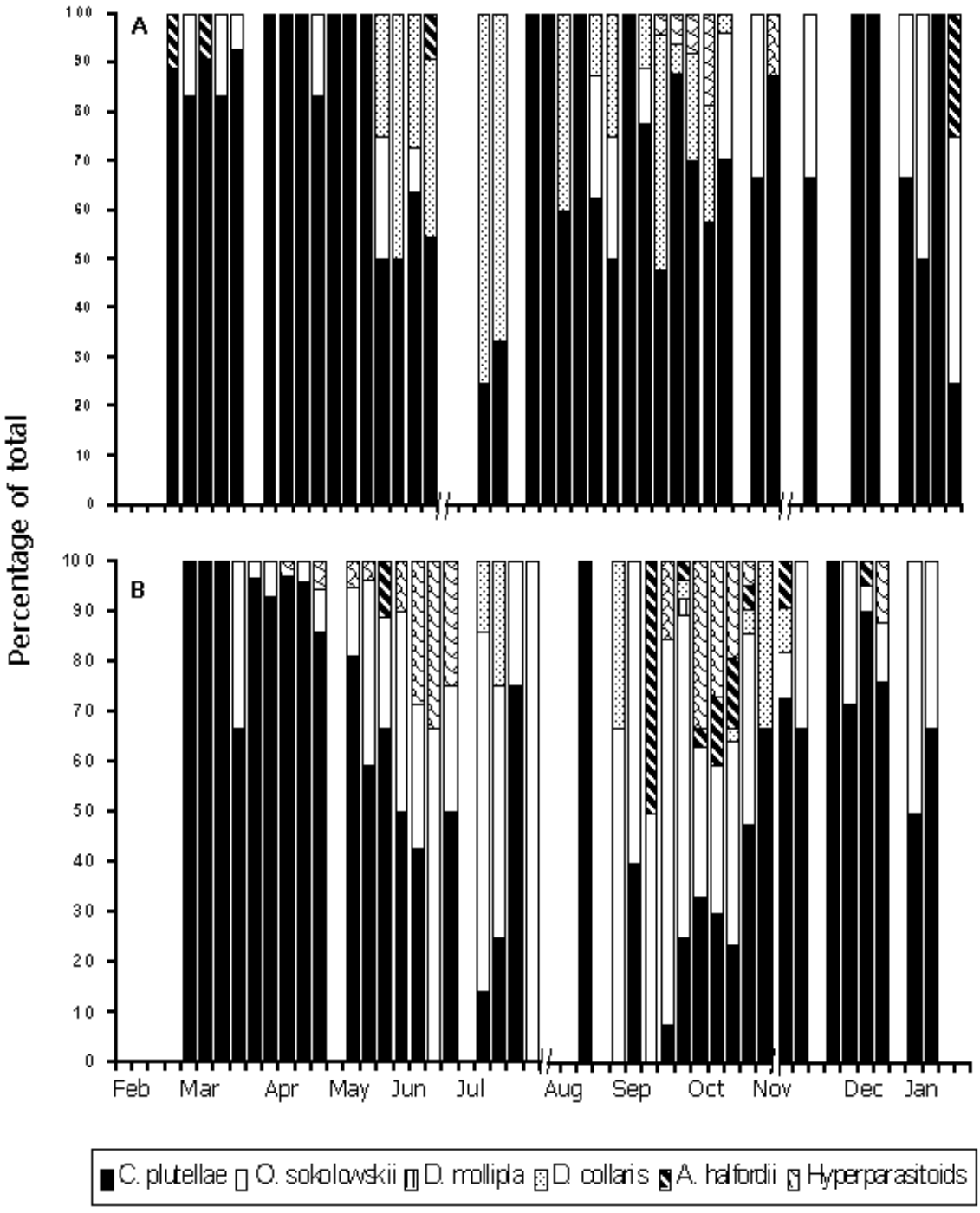

Fig. 5. 4 Composition, relative abundance and seasonality of parasitoids associated with *P. xylostella* on unsprayed cabbage plots during February 2000-January 2001 (**A**) and February 2001-January 2002 (**B**) at Brits, South Africa. Diagonal lines on the x-axis represent transplanting of new cabbage seedlings.

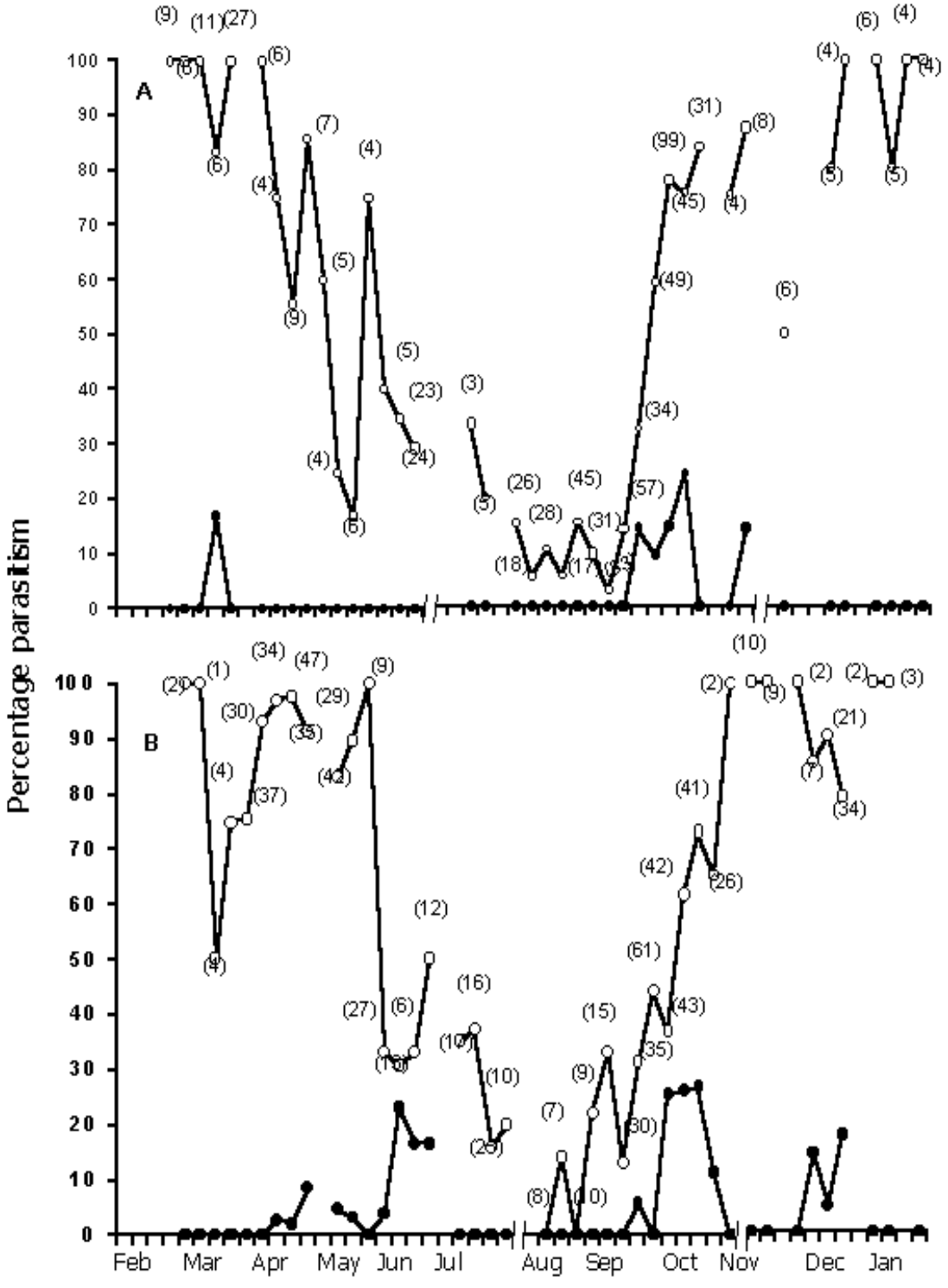

Fig. 5.5 Parasitism (open circles) and hyperparasitism (solid circles) of *Plutella xylostella* larvae during February 2000-January 2001 (**A**) and February 2001-January 2002 (**B**) at Brits, South Africa. Numbers in brackets indicate sample size. Diagonal lines on the x-axis represent transplanting of new cabbage seedlings.

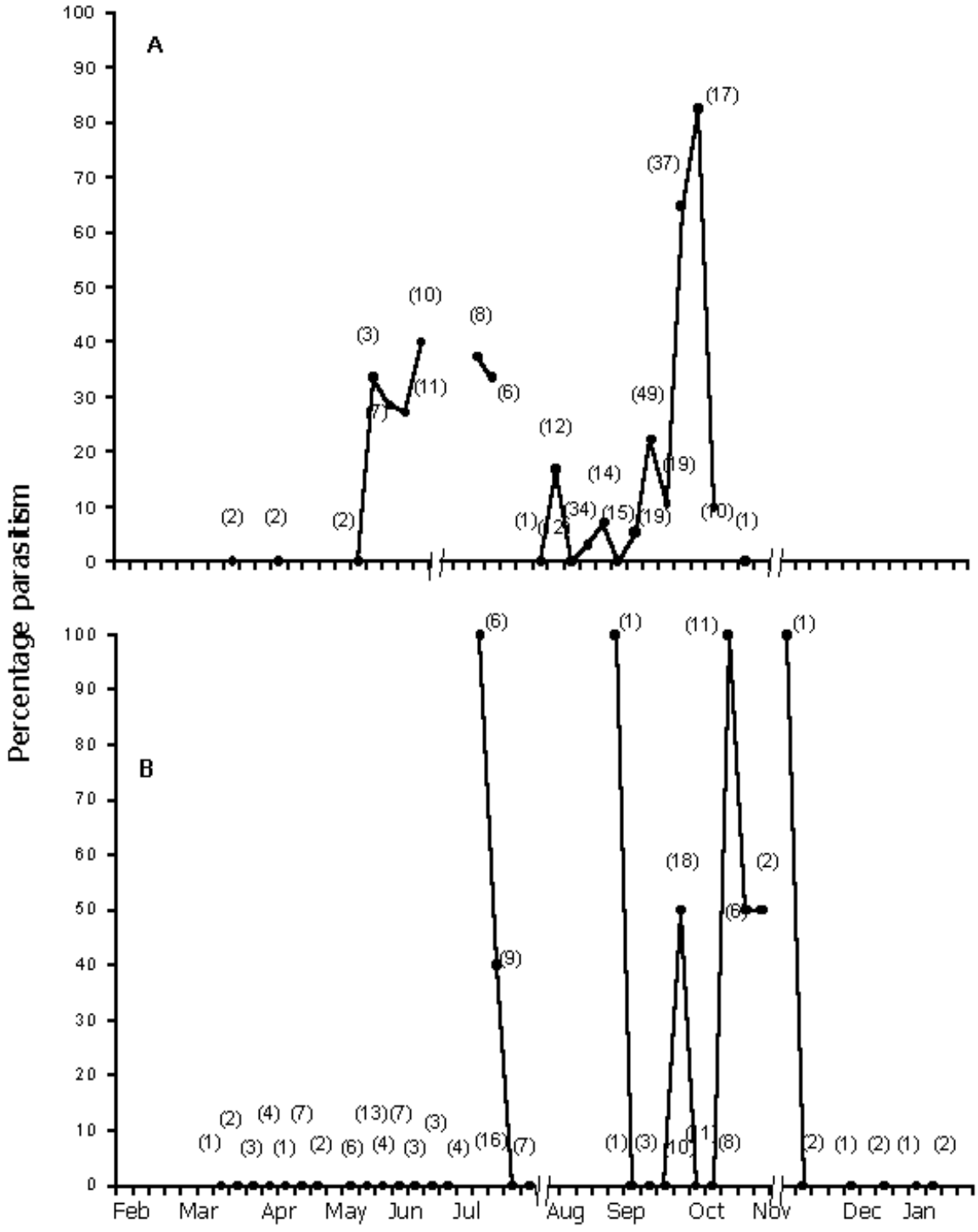

Fig. 5.6 Parasitism of *Plutella xylostella* pupae during February 2000-January 2001 (**A**) and February 2001-January 2002 (**B**) at Brits, South Africa. The numbers in brackets indicate sample size. Diagonal lines on the x-axis represent transplanting of new cabbage seedlings.

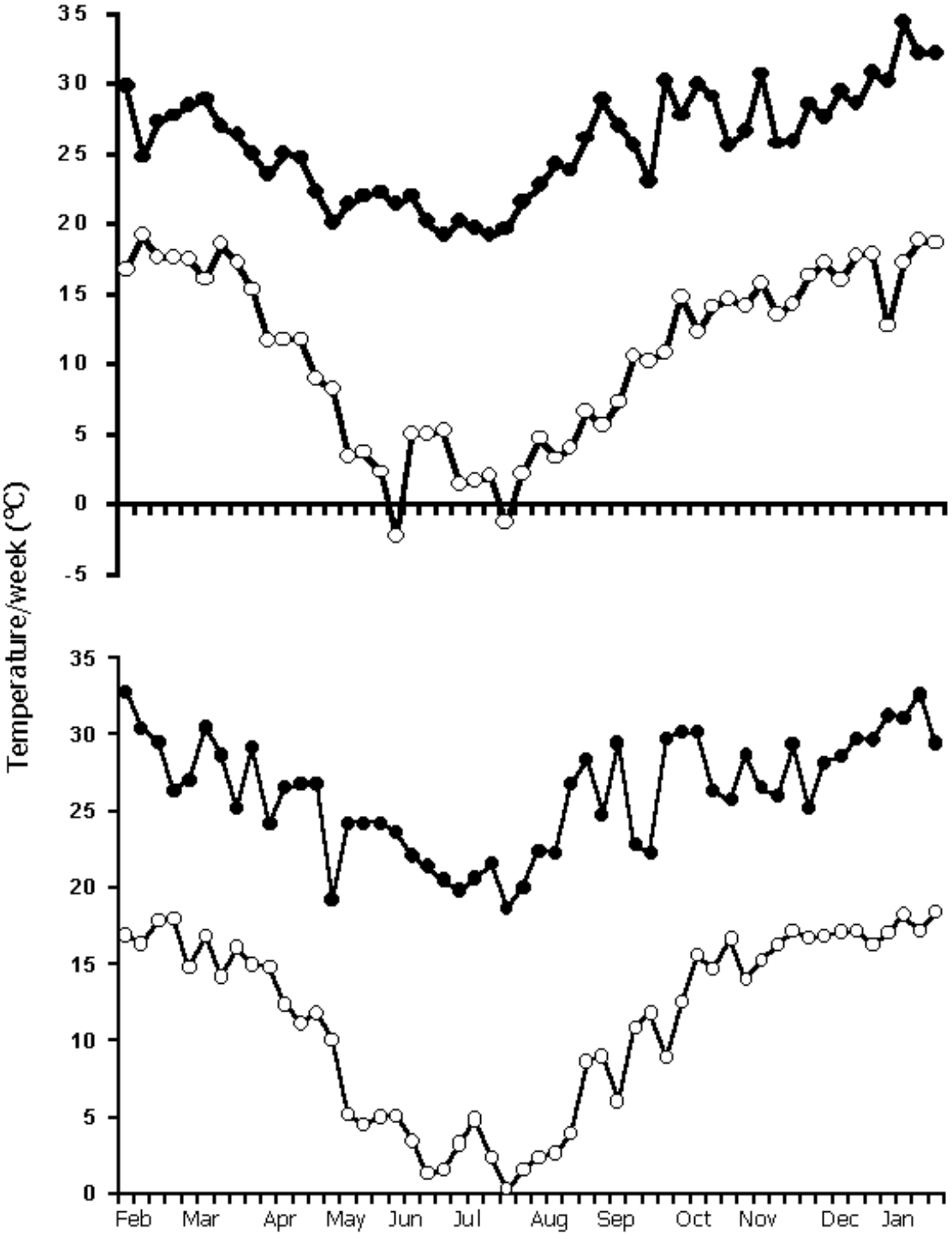

Fig. 5.7 Minimum (open circles) and maximum (solid circles) temperatures recorded during February 2000-January 2001 (above) and February 2001-January 2002 (below) at Brits-Agricultural Research Station, South Africa.

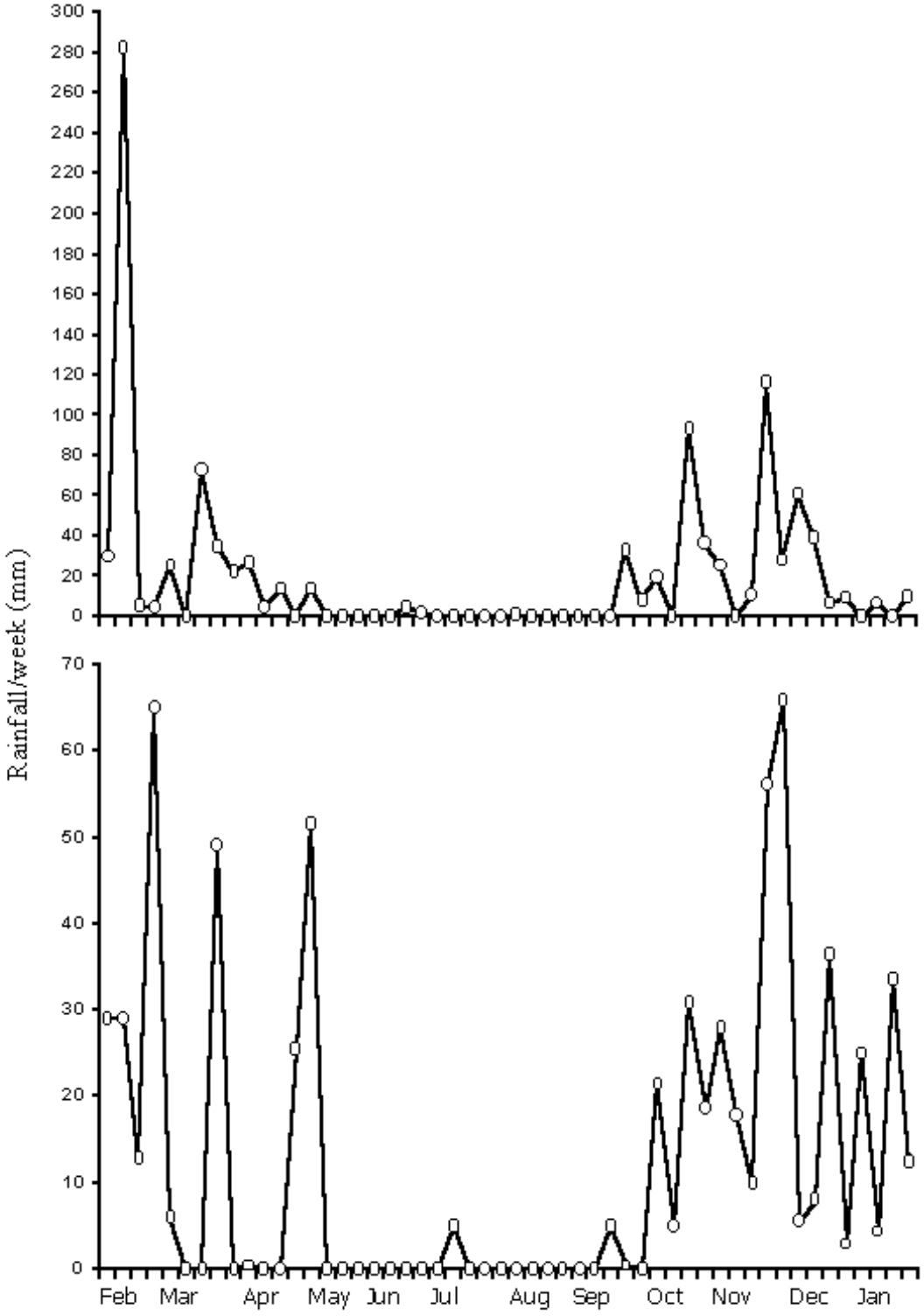

Fig. 5.8 . Rainfall recorded during February 2000-January 2001 (above) and February 2001-January 2002 (below) at Brits Agricultural Research Station, South Africa.

Chapter 6:
General discussion

In 2000, Plant Protection Research Institute (PPRI), an institute of the Agricultural Research Council in South Africa, started a collaborative project with the International Centre for Insect Physiology and Ecology in Nairobi, Kenya, on the biological control of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in Southern and East Africa. In East African countries (Kenya, Uganda, Ethiopia and Tanzania) parasitism levels of *P. xylostella* rarely are very low (<15%), and the most important parasitoids in that region are, in order of abundance, *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) and *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) (B. Löhr, ICIPE, Kenya, personal communication). The same parasitoid complex also occurs naturally in South Africa, but parasitism levels of *P. xylostella* in this country are very high usually >90% with *C. plutellae* accounting for more than 80% of total parasitism (Kfir 1997; Waladde *et al.* 2001; Smith 2002; Mosiane *et al.* 2003). Because of the higher parasitism levels of *P. xylostella* in South Africa, and the fact that *C. plutellae* is the most abundant parasitoid, it was then decided to study the biology of *C. plutellae* in the laboratory, in order to determine its suitability for release in East Africa. The biology of *D. mollipla* was also studied for comparison with *C. plutellae*. Certain aspects of their biology were studied, and these were host stage preference, fecundity, searching efficiency (all in Chapter 3), and the effects of temperature on *P. xylostella* by both parasitoids and on their rates of development and emergence (Chapter 4) In addition, the role of parasitoids in controlling *P. xylostella* on unsprayed cabbage plots was reassessed (Chapter 5).

Host stage preference experiments (*choice* and *no-choice* tests) showed that both *C. plutellae* and *D. mollipla* preferred to parasitise second and third instars more than fourth instars. If both parasitoids were to colonise a new crop in about the same densities, it is expected that that they will compete for second and third instars, as these are usually in abundance in the initial stages of crop development. Although *D. mollipla* attacked more of fourth instars than *C. plutellae*, if the latter is able to parasitise more of the younger instars than the former during the early stages of crop development, and very few individuals escape parasitism, then *C. plutellae* would always occur in very high densities than *D. mollipla.* In addition, if the data from host stage preference experiment (i.e. *choice test*) is assumed to represent the field situation, then *C. plutellae* would always occur in very high densities than *D. mollipla*, as it (unlike *D. mollipla*) produced more females per female in the younger than the older instars.

Studies on reproduction have tended to assume that a parasitoid species with higher fecundity is a better biological control agent because of its ability to kill a greater number of hosts over the course of its lifetime (Lane *et al.* 1999). In this study, *D. mollipla* produced, on average, a total of 82.57 ± 32.87 (mean ± s.d.) offspring in a life span of 7.12 ± 3.69 days whereas *C. plutellae* produced 42.13 ± 12.2 offspring in a life span of 5.23 ± 2.7 days. In nature, very few individuals live long enough to reproduce in the second half of their lives than in the first, thus natural selection would be expected to favour individuals that invest more in early reproduction even if it has an adverse effect on their potential longevity (Dixon & Agarwala 2002). Therefore, lifetime fecundity need not be the most important criterion for measuring the competitiveness of a parasitoid (Huffaker & Laing 1972), especially when both parasitoids produced about the same number of hosts in the first two days after eclosion.

Besides fecundity studies are much more useful when evaluated in conjunction with data of searching efficiency. Experiments were conducted to investigate the searching efficiency (*a*) and the killing power (*K*) of *C. plutellae* and *D. mollipla* at different host. No significant differences were observed between searching efficiencies of *D. mollipla* and *C. plutellae* ($t = -1.42$ NS, d.f. = 48, $P = 0.148$). According to Huffaker & Laing (1972) the standing or competitive ability of a parasitoid also depends on factors such as female production per female, host stage attacked, rate of development, and perhaps the ability of females to discriminate between parasitised and unparasitised hosts. In addition, adaptability to a wide range of temperatures can determine the geographical distribution of a parasitoid.

Studies were conducted on the effects of temperature on *P. xylostella* parasitism by *C. plutellae* and *D. mollipla*, and on their rates of development and emergence. Because *P. xylostella* is active throughout the year in South Africa (Kfir 1997; Mosiane *et al*. 2003) with all its developmental stages present at any given time. The reason for the dominance of *C. plutellae* over *D. mollipla* in the field can be based on factors such as its lower threshold for development, its ability to complete development at temperature as high as 33°C, its shorter generation time and high female production per female.

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