SEASONAL OCCURRENCE AND ABUNDANCE OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.), AND ITS MAJOR PARASITOIDS ON BRASSICACEOUS PLANTS IN SOUTH AUSTRALIA

by

BIJAN HATAMI

Thesis submitted for the degree

of

DOCTOR OF PHILOSOPHY

The University of Adelaide
Department of Crop Protection
Faculty of Agricultural and Natural Resources

October 1996

To my late brother

Siavash Hatami

For his spiritual inspiration and support

TABLE OF CONTENTS

		Page
LIST OF FIGURES	.....	vi
LIST OF TABLES	.....	x
SUMMARY	.....	xii
DECLARATION	.....	xv
ACKNOWLEDGMENTS	.....	xvi
LIST OF PUBLICATIONS	.....	xviii
ABBREVIATIONS	.....	xix
CHAPTER 1. Introduction.	.....	**1**
CHAPTER 2. Literature review.	.....	**4**
2.1. The pest species *Plutella xylostella* (L.).	.....	4
2.2. Parasitoids of diamondback moth.	.....	14
2.3. Measurement of parasitism.	.....	19
2.4. Management of diamondback moth.	.....	23
CHAPTER 3. Assessment of parasitism in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae).	.....	**25**
3.1. Introduction.	.....	25
3.2. Materials and methods.	.....	26
3.3. Results.	.....	34
3.4. Discussion.	.....	46

CHAPTER 4. Seasonal occurrence of *Plutella xylostella* (Lepidoptera: Plutellidae) and its parasitoids on brassicaceous weeds in the Adelaide region. **58**

4.1. Introduction. 58
4.2. Materials and methods. 59
4.3. Results. 62
4.4. Discussion. 67

CHAPTER 5. Adult activity of the diamondback moth in the Adelaide region. **73**

5.1. Introduction. 73
5.2. Materials and methods. 74
5.3. Results. 77
5.4. Discussion. 80

CHAPTER 6. Parasitism by *Diadegma semiclausum*: effects of diamondback moth larval instars. **84**

6.1. Introduction. 84
6.2. Materials and methods. 85
6.3. Results. 87
6.4. Discussion. 88

CHAPTER 7. Parasitism by *Diadegma semiclausum*: effects of host plants(cabbage, wild radish, and wild mustard). **93**

7.1. Introduction. 93
7.2. Materials and methods. 93
7.3. Results. 95
7.4. Discussion. 95

CHAPTER 8. Oviposition by diamondback moth, *Plutella xylostella* (L.)on different host plants. **98**

8.1. Introduction. 98
8.2. Materials and methods. 100
8.3. Results. 101
8.4. Discussion. 101

CHAPTER 9. Suitability of different host plants for diamondback moth. **104**

9.1. Introduction. 104
9.2. Materials and methods. 105
9.3. Results. 105
9.4. Discussion. 106

CHAPTER 10. Summary and conclusions. **108**

APPENDICES. **113**

1. Total numbers of DBM emerged and percent parasitism by *Diadegma semiclausum* during a field survey on rapeseed and kale plants at Lenswood, South Australia. 113

2. Total numbers of DBM emerged and percent parasitism by *Diadegma species* during a field survey on kale at Lenswood, South Australia. .. 114

3. Number of DBM and parasitoids based on larvae per quadrats per week and average percentage parasitism and mortality of DBM larvae at Lenswood, South Australia. 115

4. Seasonal trend and percentage parasitism of *P. xylostella* larval populations on rapeseed and kale per quadrats per week at Lenswood, SA, during the first year survey 1993-94. 116

5. Seasonal trend and percentage parasitism of *P. xylostella* larval populations on kale per quadrats per week at Lenswood, SA, during the second year survey 1994-95. 117

6. The phenology and general growth stage of more prevalent brassicaceous weeds in the Adelaide region, South Australia, 1993-95. 118

7. Number of DBM and parasitoids based on larvae per sweeping net per week and average percentage parasitism and mortality of DBM larvae in the Adelaide region, South Australia, 1994. 119

REFERENCES. **121**

LIST OF FIGURES

Page

Figure 2.1. Cabbage moth (*Plutella xylostella*). Top, the eggs and adult moth resting on cabbage leaf. Centre, a fourth instar larva on a damaged cabbage leaf. Bottom, a pupa within the cocoon. 6

Figure 2.2. *Diadegma semiclausum*, an endoparasitoid of larval cabbage moth. Top, adult. Bottom, cocoon of the parasitoid within the host cocoon. 18

Figure 3.1. Location of sampling sites for field surveys of *P. xylostella* and its parasitoids in the Adelaide region. 27

Figure 3.2. Age structure of larval *P. xylostella* from rapeseed and kale at Lenswood, South Australia 1993-1994. 35

Figure 3.3. Age structure of larval *P. xylostella* from kale at Lenswood, South Australia 1994-1995. 36

Figure 3.4. Larval density, percent parasitism and unexplained mortality of DBM based on the conventional density sampling method on rapeseed and kale at Lenswood, South Australia 1993-1994; (a) larval density of DBM; (b) percent parasitism attributed to *D. semiclausum* of three last instars (open circles) and 4th instars only (closed circles); (c) percent parasitism by *A. ippeus*; and (d) unexplained mortality. 37

Figure 3.5. Larval density, percent parasitism and unexplained mortality of DBM based on the conventional density sampling method on kale at Lenswood, South Australia 1994-1995; (a) larval density of DBM; (b) percent parasitism attributed to *D. semiclausum* of three last instars (open circles) and 4th instars only (closed); (c) percent parasitism attributed to *D. rapi* of three last instars (open circles) and 4th instar only (closed); (d) percent parasitism by *A. ippeus*; and (e) unexplained mortality. 38

Figure 3.6. Seasonal change in number of *P. xylostella* pupating per m^2, and percentage parasitism due to *B. phya*, and *D. collaris* at a kale field at Lenswood in 1993-1994 (a), and 1994-1995 (b) respectively. 42

Figure 3.7. Daily recruitment of pupal DBM and its parasitoids on kale at Lenswood, South Australia 1994-1995; (a) by *Diadegma semiclausum* and (b) *D. rapi*. 45

Figure 3.8. Schematic linkages between DBM and parasitic *Diadegma* species. 50

Figure 3.9. Percent parasitism of DBM based on the conventional density sampling and recruitment methods on kale at Lenswood, South Australia 1994-1995. Conventional density estimates were used to calculate larval parasitism on instars 2, 3, 4 together and instar 4 alone. (a) percent parasitism by *D. semiclausum*; (b) percent parasitism by *D. rapi*; and (c) DBM larval density calculated by conventional sampling and recruitment of pupae. Points for recruitment were shifted one day forward and for instar 4 one day backward to distinguish means and error bars. Error bars indicate standard error of mean. 51

Figure 3.10. Correlations between percent parasitism of DBM by *D.*

semiclausum as estimated by three different methods: conventional density sampling, recruitment, and 4th instar hosts. 53

Figure 3.11. Correlations between percent parasitism of DBM by *D. rapi* as estimated by three different methods: conventional density sampling, recruitment, and 4th larval instars. 54

Figure 4.1. Wild radish stands growing peripheral to and amongst cultivated cabbage at Virginia, a vegetable production area to the north of Adelaide, SA. 60

Figure 4.2. Densities of 2nd to 4th instar larval DBM from (a) wild radish at Lenswood; (b) wild mustard at Turretfield; (c) wild radish and wild mustard at Virginia, 1993. 63

Figure 4.3. Age structure of *P. xylostella* (a) on wild mustard at Virginia; (b) on wild mustard in the northern Adelaide Hills; (c) on wild radish at Lenswood, 1994. 65
.....

Figure 4.4. Larval densities of *P. xylostella* and % parasitism by *D. semiclausum* (a) on wild radish at Lenswood, (b) on wild mustard at Virginia, and (c) by *D. rapi* on wild mustard in the northern Adelaide Hills 1994. 66

Figure 5.1. The rope wick system for maintaining moisture in pots in the field. 76

Figure 5.2. Seasonal distribution of male DBM attracted to sex pheromone traps at Lenswood, SA. Plotted points show data Log_{10}(DBM+1)/trap/day) and solid lines are three-point moving averages. 78

Figure 5.3. Seasonal distribution of male DBM attracted to sex pheromone traps at Turretfield, SA. Plotted points show data (Log_{10}(DBM+1)/ trap/day) and solid lines are three-point moving averages. 79

Figure 5.4. Seasonal distribution of male DBM attracted to sex pheromone traps at Flagstaff Hill, SA. Plotted points show data (Log_{10} (DBM+1)/ trap/day) and solid lines are three-point moving averages. 79

Figure 5.5. Number of eggs oviposited by DBM adults on kale sentry plants, at Lenswood, SA, 1994-95. Vertical bars indicate standard error of means. The solid line is a three-point moving average. 81

Figure 6.1. Parasitism of 2nd-, 3rd-, and 4th-instars of DBM larvae by *D. semiclausum*, with separate instars or mixed instars on cabbage plants. Error bars are mean ± SE. Different letters above columns indicate statistically significant differences (P<0.05, Tukey HSD). 89

Figure 6.2. Parasitism of 1st-, 2nd-, 3rd-, and 4th-instars of DBM larvae by *D. semiclausum* in caged choice tests. Error bars are mean ± SE. Different letters above columns indicate statistically significant differences (P< 0.05, Student Newman-Keuls Test). 90

Figure 7.1. Parasitism of third instar of DBM larvae by *D. semiclausum*. The same letter at the top of each bar indicates no significant difference (P=0.05, Tukey HSD). 96

Figure 8.1. Ovipositional preferences of DBM on wild radish and wild mustard with and without the presence of crop plant (rapeseed). Values are mean ± SE. Rs, Rapeseed; Wr, Wild radish; Wm, Wild mustard. .. 102

LIST OF TABLES

Page

Table 2.1. Larval and pupal parasitoids of DBM in Australia 16

Table 3.1. Average monthly minimum and maximum temperatures (°C) and rainfall (mm) at Lenswood Research Centre, SA during 1993-1995. ... 29

Table 3.2. Sampling period of rapeseed and kale plants at Lenswood, SA 1993-1995. 30

Table 3. 3. Parasitoids reared from DBM larvae and pupae sampled from rapeseed and kale at Lenswood, SA 1993-1995. 39

Table 3.4. Total parasitism of DBM by the larval parasitoids *D. semiclausum*, *A. ippeus* and *D. rapi*, and the pupal parasitoids, *B. phya* and *D. collaris* as determined from conventional density samples taken from rapeseed and kale at Lenswood, SA 1993-1995. 40

Table 3.5. Recapture statistics for marked DBM larvae released on kale plants at Lenswood, SA. 43
.....

Table 3.6. Larval DBM developmental period and percent parasitism at constant temperature. 48

Table 4.1. Seasonal trend and percent unexplained mortality of larval DBM on weedy host plants in the Adelaide regions, SA, 1994. 68

Table 6.1. Analysis of variance for parasitism of instars of DBM larvae by *D. semiclausum.* 89i

Table 7.1. Analysis of variance for parasitism of third instar of DBM larvae by *D. semiclausum*. 96i

Table 8.1 Analysis of variance for ovipositional preferences of DBM on wild radish and wild mustard with (a) and without (b) the presence of crop plant (rapeseed). 102i

Table 9.1. Mean percentage DBM adults emerged from larvae (survival) and developmental time larval DBM reared on cabbage, wild radish and wild mustard. Values are least squares means ± SE. Values followed by the same letter in each row are not significantly different as determined by pairwise comparisons of least squares means. A total of 480 larvae were released at the start of the experiment (120/rep. × 4 replicates). 106

Table 9.2. Analysis of variance for survival (a) and developmental time (b) of larval DBM reared on cabbage, wild radish and wild mustard. 106i

SUMMARY

Diamondback moth (DBM), *Plutella xylostella* (L.) is a widespread pest of brassicaceous crops in many parts of the world and was initially reported in Australia in 1893. DBM is now the most serious threat to brassicaceous vegetables in South Australia, especially in the Adelaide region. The work described in this thesis was undertaken to quantify populations of DBM and its parasitoids, particularly the introduced parasitoid, *Diadegma semiclausum* Hellen on spring weeds and brassicaceous crops.

DBM often occurs in high numbers on brassicaceous weeds in mid spring in the Adelaide region. These DBM are from local populations. New weeds which germinate with the first rains in autumn provide a refuge for those DBM which disperse from brassicaceous crops at the end of summer. DBM simultaneously exploits brassicaceous weeds and crops from autumn to late spring according to climatic conditions. After the senescence of weeds in late spring, large numbers of DBM probably move into crops.

The initial density of DBM larvae on crops was found to be very low in early spring but increased rapidly in late spring when it peaked. Using a conventional density sampling method, it was demonstrated that relatively large numbers of larvae died from unexplained mortality factors. Second only to this, the major parasitoid *D. semiclausum* together with two other parasitoids, *Diadegma rapi* (Cameron) and *Apanteles ippeus* Nixon, were the major causes of DBM larval mortality in the field. Two pupal parasitoids were also recorded. These were *Brachymeria phya* (Walker) and *Diadromus collaris* (Gravenhorst).

Estimates of mortality caused by parasitism by *D. semiclausum* and *D. rapi* from conventional density sampling and a modified recruitment method were evaluated. Conventional density sampling was considered unsatisfactory since estimation errors were unknown and possibly large and the assumptions necessary for use of this method were not met by the populations of diamondback moth. However, parasitised larvae within the fourth instar were used to estimate the total parasitism accumulated

over all four instars. Thus the number of parasitised fourth instars could be used as an index of DBM parasitism by *Diadegma* species. The recruitment method had obvious advantages and provided an accurate estimate of ultimate percent parasitism by ichneumonid parasitoids, but neglected other associated forms of mortality such as wounding or predisposition to predation and disease. This was because total numbers of parasitised and unparasitised individuals were estimated as they entered the pupal stage. In other species, particularly pupal parasitoids, removal of pupae from field populations denied the parasitoid a resource that could otherwise be utilised. One problem with the recruitment method however, was the acquisition of sufficient numbers of cocoon samples for accurate estimates of parasitism, particularly in early spring. Also it was laborious. Percent parasitism estimated from density (conventional) samples was found to be somewhat lower than that calculated by the recruitment method in the spring and summer generations. Attempts to use trap hosts to evaluate parasitism were unsuccessful. No single method gave accurate estimates of losses due to parasitoids.

The occurrence of DBM was highly seasonal. The seasonal trend in population activity of DBM adults was estimated by pheromone trap catches. The greatest activity of moths was recorded from late August through November. The traps clearly illustrated that October was the main flight period of DBM populations in the Adelaide region of South Australia. In a supplementary field study, the relationship between DBM population densities and the number of eggs deposited on a series of kale sentry plants was investigated. The abundance of trapped males was not in conformity with oviposition by females on sentry plants.

In the laboratory, investigations were carried out to test whether different larval instars and different species of host plants influenced larval parasitism. *D. semiclausum* was found to parasitise all four larval instars of DBM, at significantly different rates: parasitism of 2nd instar was greater than all other instars and that of 4th instar was least. Furthermore, percent parasitism of 3rd instars on cabbage was significantly higher than on the weeds wild radish and wild mustard. Parasitism on the latter two, however, did not differ significantly.

Laboratory studies were undertaken to determine the effect of host plants on the oviposition, development and survival of DBM. DBM did not show a preference for the brassicaceous crop rapeseed compared to the weeds wild radish and wild mustard. The developmental time was similar on cabbage and wild mustard but one half day longer on the wild radish. Survival was the same on the three host plants.

In South Australia despite the presence of a number of natural enemies of some importance, DBM continues to maintain its pest status. Unexplained mortality (probably disease), availability of food, weather, probably immigration and emigration all play a vital role in the course of DBM populations, yet the relative contribution of its parasitoids in population control should not be underestimated.

DECLARATION

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

SIGNED: **DATE:** 5/11/96

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to all concerned and especially to the following people. I am most grateful to my principal supervisor Dr. Mike Keller for his encouragement, guidance, constructive comments and critical appraisal of this thesis. I also thank my second supervisor, Professor Otto Schmidt, for his valuable advice and friendship.

My sincere gratitude also goes to Dr. Gary Taylor and Associate Professor Rick Roush for their valuable comments on this thesis. Associate Professor Andy Austin, coordinator of postgraduate students, for his support and confirmation of identification of the parasitoid species, and Mr. Greg Baker from the South Australia Department of Primary Industries, for his friendly assistance in personal communication. I would like to thank Mr. John Crocker and other staff at the Primary Industries SA, Lenswood Centre for their cooperation in providing and maintaining the experimental plots, and Dr. Kerrie Davies and Associate Professor Stephen Powles for their friendship, moral support and encouragement.

I have been very fortunate to have made so many good friends at the Waite Institute, especially Lynne Grbin, Scott Field, Stephen Ball (whose help was above and beyond the call of duty), Jorg Kitt, Bill Frost, Pearl Field, Janine Lloyd, Nick McClure, Paul Dangerfield, Brigitte Tenhumberg, Darryl Jackman, Sassan Asgari, Hassan Alizadeh, Ebrahim Soleyman Nejadian, Latif Salehi, Hermin Herminanto, Dwi Iswari, Sheridan Purvis, Andreas Hensel, Mahmood Ahmad and Jo Kent. I cannot thank them enough for their warmth, humor, friendship and help over the past four years of my stay in Adelaide.

Many members of the Crop Protection Department assisted me during this course and I appreciate in particular Mr. Terry Feckner and Dr. Gary Taylor for cheerful managerial help, and Mrs. Anke Johnsen for her friendship and kind secretarial help on many occasions. In addition, I would also like to thank Mr. Emiel Storken and Mr. Afshin Kazerooni for their help with computer software, Ms. Emily Shephred and Ms. Sharon Clapham for their help in processing photographs and slides.

The financial support of the Ministry of Culture and Higher Education and Isfahan University of Technology, Iran, is gratefully acknowledged.

Finally, and most importantly, I am deeply indebted to my wife Mahin for her unfailing support and cheerful assistance with various stages of this project. When times were difficult, her encouragement and patience made it possible to forge on. I also appreciate my sons Saba and Sepehr, and my daughter Sarvenaz for their continual support, understanding and tolerance for the many hours I spent working on this thesis.

LIST OF PUBLICATIONS

At submission of this thesis, the following components of the study had been printed in conference proceedings:

Hatami, B. and Keller, M. A. (1994). Seasonal activity of diamondback moth and its parasitoid *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae). *Proceedings of the 25th Annual General Meeting and Scientific Conference of Australian Entomological Society.* pp. 76. The University of Adeliade, South Australia, 24-28 September.

The following paper was awarded The Phil Carne Student Prize in 1995 by The Australian Entomological Society.

Hatami, B. (1995). Comparison of two methods for assessment of parasitism in the cabbage moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Proceedings of the 26th Annual General Meeting and Scientific Conference of Australian Entomological Society.* pp. 24. Tamworth Centre for Crop Improvement, New South Wales, Australia, 23-28 September.

ABBREVIATIONS

$	Dollar
%	Percent
×	Magnification
/	Per
<	Less than
>	More than
°C	Degree Centigrade, Celsius
´	Minute
am	Before noon
Av.	Average
ca.	About
cf.	Compare with
cm	Centimeter
cv.	Cultivar
D	Dark
d	Day
DBM	Diamondback moth
diam.	Diameter
E	East
e.g.	Example given, for example
ed.	Editor
et al.	And others
h	Height
ha	Hectare
hr	Hour
i.e.	In example, that is
IPM	Integrated Pest Management
kg	Kilogram
km	Kilometer
L	Light
m	Meter
m^2	Square meter
max.	Maximum
min.	Minimum
mm	Milimeter
NE	North-east
No.	Number
pers.	Personal
pm	After noon
rep.	Replicate
SA	South Australia
SE	Standard error
sec.	Second
temp	Temperature
U.S.	United States
UC	University of California
var.	Variety
yr	Year

CHAPTER 1

GENERAL INTRODUCTION

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major and widespread pest of brassicaceous crops in many parts of the world. Waterhouse (1993) rated it a very widespread and important pest, indeed the most important species of the 159 insect pests considered in Southeast Asian countries.

Up to the 1980's DBM was readily controlled by insecticides. Since then it has increasingly developed resistance to all major classes of chemicals (Tabashnik 1986; Talekar *et al.* 1986) making it one of the most difficult pests to manage (Ankersmit 1953; Liu *et al.* 1982). Additionally, DBM was the first insect to develop resistance in the field to the bacterial insecticide *Bacillus thuringiensis* Berliner (Tabashnik *et al.* 1990). As a consequence there is increased interest in alternative methods of control (Talekar and Griggs 1986; Talekar 1992).

DBM was first reported in Australia in 1893 (Waterhouse 1992), and was an uncontrollable pest in the late 19th and early 20th centuries before the development of pesticides reduced the severity of the problem (Waterhouse and Norris 1987). In South Australia, especially in the Adelaide region, DBM is the most serious threat to brassicaceous crops and is frequently sprayed with a number of different classes of insecticides. High reliance on the use of different classes of chemicals for control has ultimately led to resistance to each type of chemical in succession (Baker 1994).

South Australia has a semi-arid climate with four-fifths of the area receiving an annual rainfall of less than 250 mm. From November (spring) to March (autumn) rainfall is generally light and variable. This low-rainfall area has mild, wet winters (June to August) and hot, dry summers (December to February). In a typical year, the opening rains arrive in autumn (April or May). Winter is usually the wettest period and crops are sown in late May to early June. Furthermore, growth of annual plants begins shortly after the first substantial rains of autumn. Growth is slow throughout winter but increases with rising spring temperatures. The lack of adequate late spring and summer rainfall in southern Australia

results in the rapid senescence of weeds and brassicaceous plants in summer and also prevents the survival of most unirrigated crops. Most weed species complete flowering and set seed by December or January. DBM often occurs in high numbers on weeds in spring. However, there is still little information on the mechanisms of long-distance migration, and short-distance dispersal of DBM amongst brassicaceous crops and weeds.

DBM feeds on members of the family Brassicaceae including cultivated crops and weeds (e.g., Harcourt 1986; Talekar and Shelton 1993; Muhamad *et al.* 1994). It has also been reported to attack several plant species from other families (Waterhouse and Norris 1987). Wild radish, *Raphanus raphanistrum* L., and the wild mustards Indian hedge mustard, *Sisymbrium orientale* L., hedge mustard, *Sisymbrium officinale* (L.), and giant mustard, *Rapistrum rugosum* (L.) are the most prevalent brassicaceous weeds in South Australia. Despite the potential for DBM population growth and maintenance associated with weeds in different geographic regions in South Australia, little is known of the extent of infestations of DBM on these alternative host plants and their relevance to population dynamics of DBM on crops in South Australia.

Parasitoids probably constitute the most abundant of all animals, possibly including 10% or more of all metazoans (Waage and Hassell 1982; Hassell and Godfray 1992), and play an important role in the regulation of their host populations (Hassell and Waage 1984; Hassell and Godfray 1992). Even though the exact role of parasitoids in the regulation of natural insect populations remains uncertain (e.g., Hassell and Godfray 1992), parasitoids have been used with varying levels of success in many biological control programs for insect pests (Huffaker and Messenger 1976; Waage and Hassell 1982; Greathead 1986).

More than 90 hymenopterous parasitoids are associated with DBM (Oatman and Platner 1969; Yarrow 1970; Putnam 1973; Goodwin 1979). DBM is parasitised by at least 10 species in Australia (Goodwin 1979). Of these, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae), a widely established and abundant parasite in many areas, is considered the most effective among the introduced species (Waterhouse and Norris 1987).

Aims

This thesis deals with the role of spring brassicaceous weeds in the population dynamics of DBM and its parasitoids. Furthermore, it examines whether factors associated with brassicaceous weeds are responsible for the larval parasitism, local distribution and mortality of DBM in brassicaceous crops. Additionally, this study aims to assess the dynamics of DBM and its natural enemies in the Adelaide region of South Australia. In the laboratory, investigations were carried out to assess the effect of host plants on the biological performance of DBM, oviposition rate and larval survival, and also to gain a better understanding of the interactions between DBM and its parasitoids.

Overview

In Chapter 2, the relevant literature that provides a general background for the present study is reviewed. The Chapters that follow describe all further observations and experiments undertaken during this study. Each is divided into a specific introduction, followed by sections detailing materials and methods, results and a discussion. Chapter 3 deals with the seasonal incidence of DBM on some brassicaceous crops and different field techniques to estimate the impact of *Diadegma* species on DBM. Chapter 4 deals with the seasonal abundance of the DBM and its major pasrasitoids on brassicaceous weeds. In Chapter 5 adult activity of the diamondback moth in the Adelaide region is investigated. The relationship between DBM population densities and the number of eggs deposited on a series of sentry (trap plants) is also discussed. In Chapter 6 the influences of different host instars on the parasitoid *D. semiclausum* are discussed. The influence of host plants on larval parasitism is tested in Chapter 7. Chapter 8 deals with oviposition preference by adult DBM on different plants. Larval survival and developmental time on different host plants is discussed in Chapter 9. A general discussion which integrates the results in the preceding chapters is presented in Chapter 10.

It should be noted that the phenology of the major brassicaceous weeds including wild radish and wild mustards is presented in Appendix 6.

CHAPTER 2

LITERATURE REVIEW

This thesis focuses on the impact of natural enemies on the population dynamics of diamondback moth. In order to understand the factors that influence populations of this insect, this chapter describes its pest status, distribution, general life cycle and development, host plants, seasonal occurrence, migration and mortality factors, and reviews the diversity of parasitoids and techniques for measuring levels of parasitism. Finally the management of diamondback moth is discussed.

2.1. The pest species *Plutella xylostella* (L.)

2.1.1. Pest status

The diamondback moth (DBM), *Plutella xylostella* (L.) (junior synonym: *P. maculipennis* (Curtis)) (Lepidoptera: Plutellidae) is a serious pest of brassicaceous crops world-wide (Bonnemaison 1965; Beirne 1971; Lin *et al.* 1984; Waterhouse and Norris 1987; Shelton *et al.* 1988). It has become the most destructive pest of brassicaceous plants in temperate regions, the tropics and subtropics (Butt and McEwen 1981; Talekar *et al.* 1985; Talekar and Griggs 1986; Talekar *et al.* 1986; Waterhouse and Norris 1987; Talekar and Yang 1991, 1993; Talekar 1992; Poelking 1992; Talekar and Shelton 1993). Indeed it is believed to be the most widely distributed of all Lepidoptera (Meyrick 1928, cited by Talekar and Shelton 1993). *P. xylostella* often needs to be controlled on cabbage, cauliflower, Brussels sprouts, broccoli and radish, and it may also damage fodder crops such as turnip and chou moellier, oil seed crops such as mustard and rape, and garden flowers such as candytuft, stock, wallflower and alyssum (Waterhouse and Norris 1987).

The absence of effective natural enemies appears to be a major cause of the pest status of DBM in most parts of the world (Lim 1986; Talekar and Yang 1993). On one hand, the more rapid establishment of DBM than its natural-enemy complex, and on the other, the use of broad-spectrum insecticides that also destroy its natural enemies contribute to the lack of effective biological control (Talekar and Shelton 1993). Indiscriminate use of insecticides, particularly in tropical to subtropical Asia, has resulted in DBM becoming resistant to many

insecticides (Miyata *et al.* 1986; Sun *et al.* 1986; Cheng 1986, 1988; Sun 1992; Talekar and Yang 1993).

The 1990 production figures of the Food and Agriculture Organisation of the United Nations (FAO) indicated that on a worldwide basis, cruciferous vegetables were grown on 2.2×10^6 ha with half this production occurring in Asia. If rapeseed acreage is added to this figure, it exceeds 17.6×10^6 ha (Talekar and Shelton 1993). The annual cost for managing DBM on these crops was estimated to be U.S. $1 billion in 1992 (Talekar 1992).

Australia is a relatively isolated continent of over 7.5 million square kilometers with two-thirds lying in the temperate zone and about a third within the tropics. Crop production areas are often dispersed and separated by considerable distances (Spooner-Hart 1993). In South Australia the total area devoted to market gardens is 10,699 ha, 4,158 of which occurs in the Adelaide district. Within this, and excluding potatoes and onions, the area planted to broccoli, Brussels sprouts, cabbages, and cauliflowers in 1992 was almost 1,107 ha with total production of 20,645 tonnes (South Australian Year book 1995). DBM is considered the most serious insect pest of these crops and they are frequently sprayed with various insecticides (Baker 1994). This (together with my field observations of high rates of insecticide application) is not in accordance with Waterhouse (1992) who indicated that DBM is seldom treated with pesticides in many areas of Australia including South Australia. He considered that DBM populations were sufficiently suppressed by a number of parasitoids including the exotic species *Cotesia plutellae* (Kurdjumov), *Diadegma semiclausum* Hellen, and *Diadromus collaris* (Gravenhorst) (Waterhouse and Norris 1987). The recent appearance of insecticide resistance in South Australia, Victoria and Queensland following years of insecticide use to control this pest indicates that Waterhouse did not correctly assess the status of this pest in Australia.

2.1.2. Life cycle and development

The eggs of DBM are minute, yellowish white to yellowish green (Figure 2.1), cylindrical to oblong with a sculptured surface (Marsh 1917; Hardy 1938), generally laid singly or in groups of two to four often along the mid-ribs or principal veins on the undersides of leaves (Bhalla and Dubey 1986), on indented surfaces near smaller veins (Gupta and Thorsteinson 1960b; Chelliah and Srinivasan 1986), or in groups of up to eight, mainly on the upper

Figure 2.1. Cabbage moth (*Plutella xylostella*). Top, the eggs and adult moth resting on cabbage leaf. Centre, a fourth instar larva on a damaged cabbage leaf. Bottom, a pupa within the cocoon.

surface of leaves (Waterhouse and Norris 1987). In the laboratory, caged adult females deposit eggs on different parts of potted plants, including the stems. Oviposition is stimulated by a reduction of light during normal daylight, but it is not inhibited by light during night hours (Tabashnik 1985; Talekar and Shelton 1993). The incubation period is highly influenced by temperature (Talekar and Shelton 1993), with a minimum of 3 days (Marsh 1917; Vos 1953).

Newly hatched larvae are whitish yellow to pale green with a pale brown head (Bhalla and Dubey 1986; Chelliah and Srinivasan 1986). Mature larvae are pale-green, slightly tapered at each end, and measuring up to 10 mm in length (Figure 2.1). They wriggle rapidly when disturbed, often dropping from the plant on a silken thread. First instar larvae are leaf-miners while older larvae, i.e., beyond the beginning of the second instar, feed by scraping the epidermis of leaves, preferentially the younger leaves in the middle and inner part of the host plant (Harcourt 1957; Ooi 1986; Talekar and Shelton 1993). DBM has four instars, the duration of which depends on temperature (Bhalla and Dubey 1986; Salinas 1986; Sarnthoy *et al.* 1989; Talekar and Shelton 1993). Each instar can be distinguished by the width of the head capsule (Herminanto 1995). Generally, the duration of the larval period varies from 9 to 30 days (Waterhouse and Norris 1987).

Larvae construct a loosely spun cocoon and spend a two-day period of quiescence marking the prepupal stage. Initially the newly formed pupa is yellowish green (Figure 2.1), but in a day or two it becomes brownish and gradually assumes a dark brown colour by the time of adult emergence (Bhalla and Dubey 1986). The duration of the pupal period depends on temperature and varies from 4 to 15 days (Harcourt 1957; Chelliah and Srinivasan 1986; Talekar and Shelton 1993).

The adult is a slender, greyish-brown moth which, in resting has a longitudinal creamy-yellow dorsal band with three constrictions (Figure 2.1). This produces distinctive diamond shapes giving the moth its common name (Waterhouse and Norris 1987). Activity of DBM adults commences at dusk and lasts into the night (Bhalla and Dubey 1986; Poelking 1992; Talekar and Shelton 1993). The majority of adults emerge during the first 8 hr of photophase (Pivnick *et al.* 1990a). Adults mate at dusk, usually on the day of emergence (Harcourt 1957; Chelliah and Srinivasan 1986; Taleker and Shelton 1993). Females may mate only once (Harcourt 1957), but repeatedly mate under laboratory conditions (Moller

1988). However, oviposition may begin on the day of emergence (Harcourt 1957) or immediately after copulation (Moller 1988). The male to female sex ratio in the DBM is virtually 1:1 (Harcourt 1957, 1986).

The life cycle from egg to adult stage of the female and male varies according to environmental factors. Although 17 to 25°C is considered the optimum temperature range of DBM (Atwal 1955), it has an ability to survive in a wide range of temperatures (Ooi 1986). At high temperatures the life cycle may be as short as 16 days (Waterhouse and Norris 1987). At different estimated threshold temperatures for the development from egg to adult, the required thermal accumulation varies. For example, the development from egg to adult was reported to require 283 day-degrees with a threshold of 7.3°C (Harcourt 1954, cited by Baker *et al.* 1982), but other authors have reported different developmental parameters (e.g., Butt and McEwen 1981; Sarnthoy *et al.* 1989; Choi *et al.* 1992). It appears that geographic variation in the developmental response of DBM to temperature is common. Developmental period and adult longevity are not significantly related to photoperiod (Shirai 1993). There is no significant difference in the longevity of female and male DBM adults; both range from seven to nine days at 22.5°C (Atwal 1955).

A major factor contributing to changes in numbers of insects between generations can be the failure of populations to realize their potential fecundity (Bellows *et al.* 1992). Females lay up to 360 eggs (Harcourt 1957); the mean number of eggs deposited per female is quite variable but peak numbers are deposited in the first night of adult life, following a preovipositional period of less than one to nearly two days (Muhamad *et al.* 1994). The ability of adult female to deposit their full complement of eggs or offspring may depend on the effects of weather (Courtney and Duggan 1983), changes in plant characteristics such as those due to water stress (Preszler and Price 1988), levels of soil nutrients, adult emigration or the direct action of natural enemies (Bellows *et al.* 1992). The temperature under which DBM is reared affects its fecundity (Koshihara 1986). For example, females reared at low temperatures (7 to 24°C) lay more eggs than those reared at higher temperatures (28 to 35° C) (Atwal 1955). A positive correlation was observed between the longevity and fecundity of females (Poelking 1992). The length of photophase during larval development also affects fecundity. Atwal (1955) reported an increase in fecundity when hours of daylight were increased from nine to fifteen. The quality of food affects the development and

fecundity of DBM (Koshihara 1986). Harcourt (1986) indicated that the fecundity of DBM was density independent, and directly related to the level of crude protein of the food plant which decreased gradually throughout the cropping season. DBM has a lower fecundity on some brassicaceous weeds (Wakisaka *et al.* 1992; for an overview see: Muhamad *et al.* 1994).

2.1.3. Male diamondback moth response to sex pheromone

Chow and his co-workers isolated the sex attractant pheromone of the DBM in 1974. The components of pheromone blend has been identified as (Z)-11-hexadecenal, (Z)-11-16-hexadecenyl acetate and (Z)-11-hexadecen-1-o1 (Maa 1986). Studies on pheromone composition of DBM have shown that variations of adult male response to the synthetic sex pheromone depends on the ratio of the components of the pheromone (Maa 1986), and on their concentration (Chisholm *et al.* 1979; Unal *et al.* 1993). Furthermore, temperature and seasonal variations affect male moth response to the sex pheromone (Maa 1986).

Pheromones are used to monitor field populations of DBM (Chisholm *et al.* 1979; Baker *et al.* 1982; Hallett *et al.* 1993). In population sampling generally, and in surveying DBM populations in particular (Koshihara 1986), the use of pheromone traps is particularly useful because it allows for continuous sampling of populations and attraction of only one species usually occurs (Daly and Fitt 1993). Due to lack of side effects, sex pheromones should also be considered as an alternative to insecticides for the management of DBM. Mclaughlin *et al.* (1994) reported the successful use of pheromones in mating disruption to suppress a DBM population in Florida. Furthermore, in Japan, Ohno *et al.* (1992) reported that pheromone mating disruption reduced populations of DBM by 95%.

2.1.4. Host plants

Host plants of DBM include a large number of species mostly in the family Brassicaceae (Thorsteinson 1953; Harcourt 1957, 1986; Gupta and Thorsteinson 1960a; Horn 1987; Poelking 1992; McLaughlin *et al.* 1994). These hosts include cultivated crops and a variety of weeds of this family and sometimes others (Harcourt 1986; Talekar and Shelton 1993; Muhamad *et al.* 1994). In spring before the brassicaceous crops are planted, wild host plants play an important role in maintaining DBM populations in temperate regions (Talekar and Shelton 1993). Furthermore, it has been observed that in the absence of favoured cultivated crops during the summer, DBM depends on less suitable host plants such as Shepherd's

Purse, *Capsella bursa-pastoris* (for an overview see: Muhamad *et al.* 1994). Weeds are major components of agro-ecosystems that affect the biology of pests and beneficial insects in several ways (Altieri *et al.* 1977). Natural enemies can be more effective and abundant in weedy plots (Root 1973), and some parasitoids (e.g., Ichneumonidae) depend on weeds (Syme 1975). Furthermore, the presence of a variety of insects on weeds may serve as alternative food for predators or parasites (Altieri *et al.* 1977).

The presence of mustard oils and their glucosides, compounds characteristic of the Brassicaceae, influence the susceptibility of host plants to DBM. These chemicals are utilized by DBM larvae as phagostimulants (Gupta and Thorsteinson 1960a, b; Hillyer and Thorsteinson 1971; Talekar and Shelton 1993) and by adults as oviposition stimulants (Nielsen 1978, 1989; Reed *et al.* 1989; Olsson and Jonasson 1994). The presence of unidentified olfactory stimuli attract DBM to brassicaceous plants (Palaniswamy *et al.* 1986; Pivnick *et al.* 1990b).

Habitat has been defined by Huffaker and Rabb (1984) as "the physical area encompassing the resources which support the existence of an individual insect or insect population for a specified period of time". DBM lives in habitats of brassicaceous plants including cultivated plants and weeds.

Colonisation and utilisation of a host plant by its herbivores and the level of herbivory that a host plant bears are influenced by many factors (Letourneau and Fox 1989). The spatial and temporal availability of host plants, and behaviour and abundance of enemies and competitors of an herbivore determine its colonisation behaviour (Cromartie 1975). Plant diversification influences the population dynamics of insect herbivores, in agricultural (Root 1973; Risch *et al.* 1983; Andow 1988) and natural habitats (Kareiva 1983; Stanton 1983). Furthermore, the spatial arrangement of plants influences their associated insect community (Root 1973; Risch 1979; Gould and Stinner 1984). Phytophagous insects often attain highest densities where their food plants occur in large monospecific patches (e.g., Cromartie 1975; Thompson 1978; MacGarvin 1982). A vegetative (weedy) background significantly decreases colonisation of collard plants (*Brassica oleracea* L.) by herbivorous insects (Pimentel 1961a; Cromartie 1975). The presence of vegetation surrounding a plant could lower that plant's susceptibility to herbivory (Root 1973). In other words, plants that grow in diverse natural vegetation may possess an associational resistance to their

specialised herbivores, and outbreaks of these species are more likely to occur in monocultures (Tahvanainen and Root 1972; Root 1973). The presence of weeds among crop plants affects colonisation of phytophagous insects and subsequently the rate of predation and parasitism (Root 1973; Horn 1987). Generally, colonisation of host plants by specialist phytophagous insects is reduced by weedy vegetation while it provides alternate food and buffers extreme temperature and humidity for generalist predators and parasitoids (Horn 1981). Additionally, wild hosts play an important role in maintaining a population of pest insects, particularly before the planting of crops (Harcourt 1986; Horn 1987). In tropical and subtropical areas large pest populations can be promoted by continuous or year-round production of a single crop in a given area (Huffaker and Rabb 1984).

Plant density affects herbivore populations in several ways (e.g., Pimentel 1961b; Way and Heathcote 1966; Ralph 1977; Thompson 1978). Differences in plant density strongly influence the quality and quantity of host plants available (e.g., Harper and McNaughton 1962; Way and Heathcote 1966; White and Harper 1970; Ford 1975). Herbivore growth depends on the quality and quantity of available host plants (e.g., Chew 1975; Scriber and Slansky 1981). It has been concluded that host plant quality is an important factor driving the population dynamics of herbivores like DBM (Letourneau and Fox 1989). With DBM it is evident that quality of food influences its development and fecundity (Koshihara 1986). Adult size and flight ability of DBM are also influenced by food during the larval period (Muhamad *et al.* 1994). Andow *et al.* (1986) reported that insect population densities were lower on cabbage grown with natural mulch than in bare-ground monocultures. Furthermore, DBM larval densities were influenced directly by non-host neighbor plants rather than by indirect effects on host plant size or quality (Bach and Tabashnik 1990). In certain crops pest susceptibility is influenced by fertilisation regimes (Huffaker and Rabb 1984). Accordingly, parasitoid and DBM abundance were related to the total leaf nitrogen and female-biased sex ratios of the wasps were correlated with higher nitrogen concentrations in the foliage consumed by DBM (Fox *et al.* 1990). According to Talekar and Yang (1991) parasitism by *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) was greater when DBM larvae were feeding on common cabbage (*Brassica oleracea* var. *capitata* L.), than on cauliflower (*B. oleracea* var. *italica* L.), broccoli (*B. oleracea* var. *botrytis* L.), or Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis* (Lour) Olsson).

2.1.5. Seasonal occurrence and migration

DBM is a multivoltine species that breeds continually, with a number of generations per year. There are many more generations of DBM in tropical areas than in temperate regions. In temperate climates DBM completes 4-6 generations a year (Harcourt 1963; Tabashnik *et al.* 1987), and many more in the tropics, e.g., 15-20 generations per year in Hawaii (Caprio and Tabashnik 1992). In high latitudes (e.g., Canada) DBM is believed not to overwinter and its populations are thought to die out completely each year to be replaced in spring by migrants from the south (Harcourt 1957; Butt and McEwen 1981).

There is no evidence that DBM can diapause or hibernate in any life stages (Atwal 1955; Harcourt and Cass 1966; Yamada and Umeya 1972). Many studies have shown that no DBM could be collected during the coldest periods (Honda *et al.* 1992; Talekar and Shelton 1993). In contrast, all stages of DBM are present at any time in the tropics and subtropics where brassicaceous plant growth is observed throughout the year.

The migration of DBM is an important factor in its distribution (Chu 1986; Talekar and Shelton 1993). Therefore, to establish a valid control program, an understanding of the dispersal distance of DBM or of factors influencing its flight behavior on crop fields is important (Shirai and Nakamura 1994). Numerous reports are available on the ability of DBM to migrate long distances. For example, in Europe DBM has been shown to migrate over distances in excess of one thousand kilometers in a storm caused by low atmospheric pressure (French and White 1960; Honda *et al.* 1992), or adults migrate from the Baltic countries and southern Finland, a distance of more than 3,000 km to initiate its annual occurrence in Britain (Mackenzie 1958; Talekar and Shelton 1993). In the Orient, there is considerable variation in DBM population densities at various geographical locations. Here, mass migration has not been directly observed, but a few DBM have been captured over the East China Sea in excess of 500 km from the nearest major landmass (Chu 1986). In other parts of the world, e.g., Japan (Yamada and Umeya 1972; Honda 1992), New Zealand, South Africa, southern parts of Chile and Argentina, and Australia similar migrations of DBM may occur (Talekar and Shelton 1993). In South Australia, local populations in combination with DBM that migrate from low rainfall areas probably cause damage on crops from mid spring ownward in the Adelaide region (G. Baker 1995, pers. communication).

2.1.6. Mortality factors other than parasitism

DBM is attacked by a combination of density-dependent and density-independent mortality factors that influence its population size. Weather is density independent and is the principal factor that causes instability in the DBM life system (Harcourt 1986). In temperate regions, high temperatures in the summer (Yamada and Umeya 1972) in conjunction with the disappearance of almost all cruciferous weeds during this period (Koshihara 1986) cause the remarkable summer decline of DBM populations. DBM peaks during spring when climatic conditions are not hot and dry. The activity of natural enemies affects the seasonal fluctuation of DBM (Iga 1985). Apart from parasitism which is a major mortality factor of DBM larval and pupal stages, physiological death (i.e., unhatched eggs; Wakisaka *et al.* 1992), and predation by polyphagous predators such as birds, spiders, and some other arthropods are also implicated as major mortality agents (Chelliah and Srinivasan 1986; Wakisaka *et al.* 1992). Rainfall (Harcourt 1963, 1986; Talekar *et al.* 1986; Chelliah and Srinivasan 1986; Waterhouse and Norris 1987; Sivapragasam *et al.* 1988; Wakisaka *et al.* 1992; Poelking 1992) and disease (Wakisaka *et al.* 1992; Poelking 1992; Riethamacher and Kranz 1994) are direct mortality factors that also severely affect survival of young larvae. Larval mortality under moist conditions by the fungal pathogen, *Entomophthora sphaerosperma* Fres. has also been reported (Waterhouse and Norris 1987; Riethamacher and Kranz 1994).

2.2. Parasitoids of diamondback moth

2.2.1. Diversity of parasitoids

Remarkable biological and taxonomic diversity is exhibited by parasitoids (Waage and Hassell 1982). Askew (1971) estimated that there are over 100,000 species of parasitic Hymenoptera worldwide. It is common to find parasitoid genera containing over 1000 species, a diversity which poses considerable problems for taxonomists (Waage and Hassell 1982). The number of parasitoid guilds on a host relates to the number and duration of distinct host stages. Furthermore, the species composition of parasitoid guilds varies over the geographical and host plant range of a host (Waage and Hassell 1982).

Over 90 species of hymenopterous parasitoids are associated with eggs, larvae, and pupae of DBM (Thompson 1946; cited by Cordero and Cave 1992; Oatman and Platner 1969;

Yarrow 1970; Putnam 1973; Goodwin 1979; Lim 1986). However, not all parasitoids are effective against DBM. Among them only about 60 appear to be important (Talekar and Shelton 1993). Most of these parasitoids originate from Europe, including the two major parasitoid genera *Diadegma* and *Cotesia*. Those parasitoids of DBM that have the highest control capability belong to the genera *Microplitis* (Lim 1986), *Diadegma*, *Cotesia* and *Apanteles* (Waterhouse 1992). Among these, *D. semiclausum* is one of only three species successfully introduced in areas where they did not originally exist (Yang *et al.* 1993). Some parasitoids are heavily attacked by hyperparasitoids.

Diadegma is a very large and taxonomically difficult genus of the Ichneumonidae (see table 2). Fitton and Walker (1992) reported nine *Diadegma* species which attack DBM. Indeed, other workers have reported up to twelve species in a given locality. For example, in Romania, Mustata (1992) reported twelve species of *Diadegma* among the complex of parasitoid and hyperparasitoid species associated with DBM, and indicated that *D. fenestralis* Holmgren was one of the major parasitoids.

In Australia the following species have been reported by Yarrow (1970) and Goodwin (1979). *D. semiclausum* (misidentified as *Diadegma cerophaga* (Gravenhorst)) (Fitton and Walker 1992) was originally imported from Europe and released in Queensland in 1947 (Yarrow 1970) and *Diadegma rapi* (Cameron), is an apparently indigenous Australian species (Gauld 1984). *D. semiclausum* and *D. rapi* are morphologically distinct (Gupta 1964). *D. rapi* is recognised easily because it lacks one small cross-vein, 3*r-m* or areolet (the 2nd submarginal cell) in the forewing (Gauld 1984; Fitton and Walker 1992). Venkatraman (1964) in a laboratory study in Australia investigated interrelationships between *D. semiclausum* and *D. rapi* and stated that these two species were closely related to each other, and that they copulate in the insectary. In the present study, copulation between the two species *D. semiclausum* and *D. rapi* has been observed on two occasions. However, the species level taxonomy of the genus *Diadegma* is in desperate need of revision in Australia. Other parasitoids of DBM recorded from Australia are presented in Table 2.1. The references in the table have been abridged to include only those which contain detailed biological information. Among these parasitoids the more important ones

Table 2.1. Larval and pupal parasitoids of DBM in Australia.

Current name	Family	Junior synonyms	Type†	Stage attacked	References
Antrocephalus sp.	Chalcididae		P	Larva	(Goodwin 1979)
**Apanteles ippeus* Nixon	Braconidae		P	Larva	(Wilson 1960; Yarrow 1970)
Cotesia plutellae (Kurdjumov)	Braconidae	*Apanteles plutellae* Kurdjumov	P	Larva	(Wilson 1960;Yarrow 1970)
**Diadegm semiclausum* Hellen	Ichneumonidae	*Angitia cerophaga* (Gravenhorst) *Diadegma cerophagus* (Gravenhorst) *Diadegma eucerophaga* Horstman *Horogenes cerophaga* (Gravenhorst) *Horogenes fenestralis* Holmgren *Horogenes tibialis* (Gravenhorst) *Nythobia cerophaga* (Gravenhorst)	P	Larva	(Wilson 1960; Yarrow 1970; Goodwin 1979)
**Diadegma rapi* (Cameron)	Ichneumonidae	*Hymenobosmina rapi* (Cameron)	P	Larva	(Wilson 1960; Goodwin 1979)
Diplazon laetatorius Fabricius	Ichneumonidae		P	Larva	(Yarrow 1970)
Dolichogenidea sp.	Braconidae	*Apanteles laevigatus*-group	P	Larva	(Goodwin 1979)
Spinolia sp.	Ichneumonidae	*Chirotica* sp.	P	Larva	(Goodwin 1979)
Stictopisthus sp.	Ichneumonidae		P	Larva	(Goodwin 1979)
**Brachymeria phya* (Walker)	Chalcididae	*Brachymeria plutellophaga* (Girault) *Brachymeria victoria* (Girault) *Chalcis victoria* (Girault)	P	Pupa	(Yarrow 1970; Boucek 1988)
Brachymeria sidnica Holmgren	Chalcididae	*Chalcis tegularis* Cameron *Chalcis multicolor* Girault *Chalcis thymus* Girault *Chalcis silvae* Girault *Chalcis elijahi* Girault *Pseudepitelia tricolor* Girault	P	Pupa	(Yarrow 1970; Boucek 1988)
**Diadromus collaris* (Gravenhorst)	Ichneumonidae	*Thyraeella collaris* Gravenhorst	P	Pupa	(Wilson 1960; Yarrow 1970)
Ceraphron fijiensis Ferris	Ceraphronidae		H	?	(Yarrow 1970)
Eupteromalus sp.	Pteromalidae		H	?	(Yarrow 1970; Goodwin 1979)
Lienella sp.	Ichneumonidae		H	?	(Yarrow 1970)

*: Parasitoids which were found during this study; P†: Primary parasitoid; H: Hyperparasitoid.

are *D. semiclausum*, *D. rapi*, *Diadromus collaris*, *A. ippeus*, and *C. plutellae* (Wilson 1960; Goodwin 1979).

2.2.2. Biology of *D. semiclausum*

D. semiclausum (Figure 2.2), a solitary endoparasitoid, is host specific to *P. xylostella* (Gauld 1984) and native to Europe (Waterhouse and Norris 1987). Under laboratory conditions at 25°C, Abbas (1988) reported that the incubation period of eggs lasted 38 hr, the five larval instars 5.4 d, the pre-pupa 33 hr, and the pupa 5.9 d. Furthermore, the daily and total egg deposition were 13.6 and 164.2 per female, respectively. Development and fecundity are highly dependent on temperature. Emergence of the larva of *D. semiclausum* is from the host prepupa and construction of its own cocoon occurs inside the host's cocoon (Figure 2.2).

Parasitism by *D. semiclausum* is highest in cooler temperate regions or only in the highlands of the tropics. This occurs because there is a drastic reduction of parasitism *by D. semiclausum* above 30°C (Talekar and Yang 1991; Yang *et al.* 1993; Herminanto 1995).

Although many ichneumonids diapause through winter as full-grown larvae within their cocoons, it is not known how *Diadegma* overwinters in temperate zones (Fitton and Walker 1992). Winter ploughing in many crops destroys *Diadegma* cocoons but sometimes high rates of parasitism of DBM in spring are explained by a large overwintering population on other brassicaceous plants (Fitton and Walker 1992). Despite the ability of DBM for migration, no record of migration of any of its parasitoids is available (Talekar and Shelton 1993).

Adults of *Diadegma* feed from flowers, and levels of parasitoid activity in crops depend upon the availability of suitable flowers in and around crops (Fitton and Walker 1992).

Host selection by a parasitoid is under the effect of both physical and chemical factors (Vinson 1976; Sands 1993). Most species of *Diadegma* are relatively host-specific and the usual hosts are microlepidoptera (Fitton and Walker 1992). Abbas (1988) failed to rear *D. semiclausum* on a variety of lepidopterous larvae other than DBM in laboratory trials, indicating that it is specific to DBM. Although all instars are parasitised, the second and

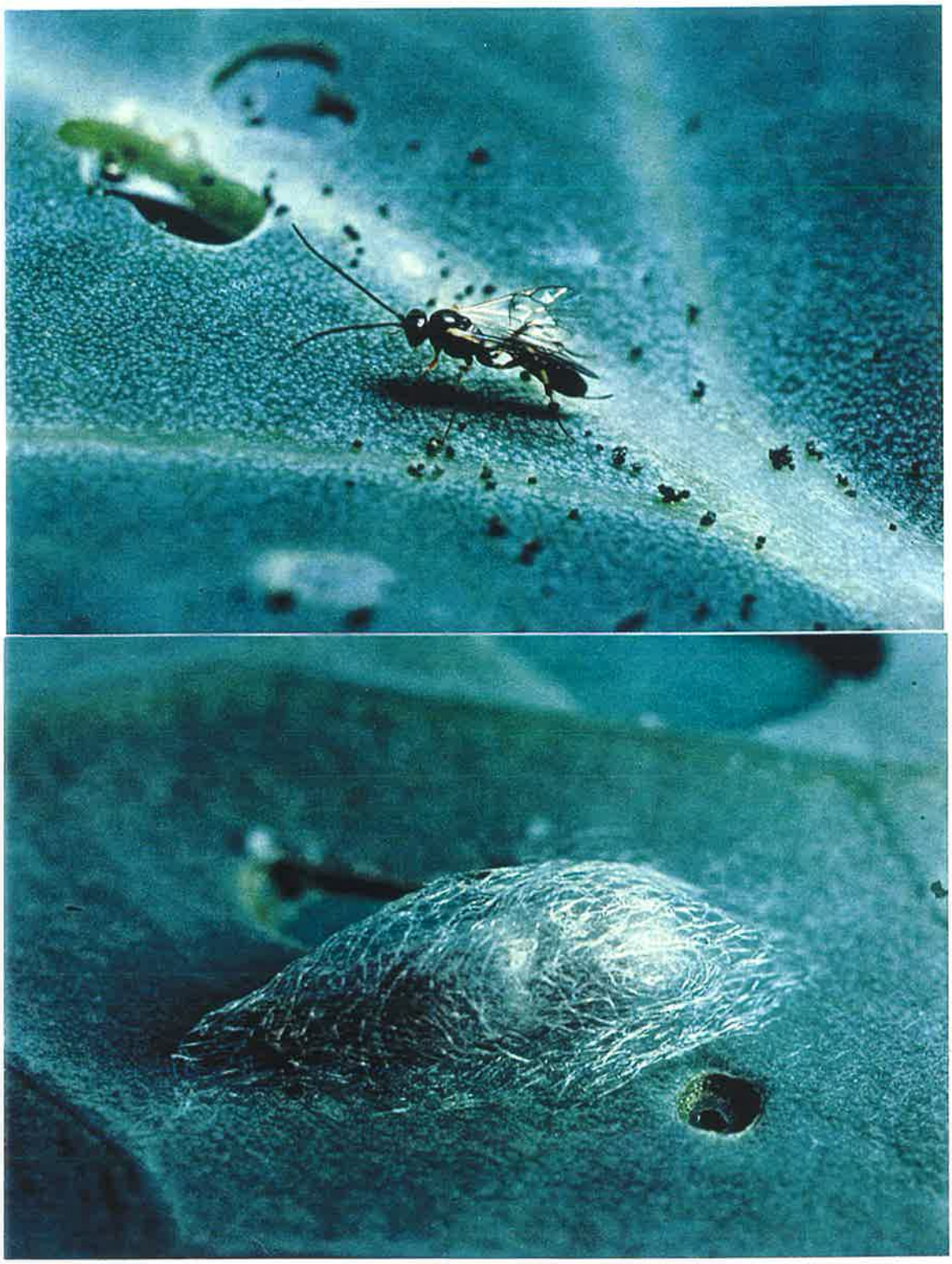


Figure 2.2. *Diadegma semiclausum*, an endoparasitoid of larval cabbage moth. Top, adult. Bottom, cocoon of the parasitoid within the host cocoon.

third instars are preferred (Lloyd 1940; Velasco1982). In the case of *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae), no parasitoids emerged from DBM that were offered to ovipositing wasps as 1st instars, but 26-36% of the larger instars were parasitised (Fox *et al.* 1990).

Most of the reproductive activities of *D. semiclausum*, such as mating and oviposition seem to be stimulated by daylight (Yang *et al.* 1993), and DBM larvae are parasitised by *D. semiclausum* only during daytime (Talekar and Yang 1991).

Sex ratios affect parasitoid-host interactions because only females are host foragers, and this sex-allocation to progeny directly affects the numerical response (Fox *et al.* 1990). In this context, *Diadegma* spp. have variable sex ratios (Fox *et al.* 1990), however, in *D. semiclausum* they are strongly male-biased (ca. 25% female). This was considered a factor in preventing it from regulating populations of DBM (Chua and Ooi 1986; Yang *et al.* 1993). Waage (1982) in laboratory experiments found that sex ratios varied from 24-68% female, influenced by both abiotic and biotic factors (Waage and Hassell 1982). Fox *et al.* (1990) showed that the sex ratio of a solitary ichneumonid wasp, *Diadegma insulare* Cresson varied markedly in response to the quality of its host (DBM larvae), and apparently to the differences in the quality of the host-plants.

2.3. Measurement of parasitism

2.3.1. Assessment of parasitism

The accurate measurement of insect mortality caused by parasites and disease is fundamental to biological control, ecology, and related fields (Day 1994). For measuring insect mortality by parasites and disease, two methods, rearing and dissection, are most frequently used. However, many studies have shown that parasitism measured by dissection was higher than by rearing, and that dissection is much more accurate than rearing, so they are not likely to be of equal value in measuring insect mortalities (Day 1994). Waage and Mills (1992) noted that the assessment of the impact of natural enemies was generally based on their relative abundance. When parasitism of an insect population is evaluated, the ratio of the number of parasitised insects to the combined numbers of parasitised and healthy hosts in the sample is reported commonly as the "percent

parasitism". In most instances, parasitoid impact has been measured by this method (van Driesche 1983; Waage and Cherry 1992). In insects, such as DBM, which have overlapping recruitment and losses, estimated densities and percentage parasitism values do not always measure adequately the effect of parasitoids (van Driesche *et al.* 1991). Waage and Mills (1992) pointed out that impact of natural enemies from limited sampling of the pest must be interpreted with care. van Driesche (1983) illustrated how observed values of percent parasitism were influenced by phenological processes, thus making such samples poor indicators of actual parasitoid impact on hosts. Lopez *et al.* (1993) reported that percent parasitism from density samples using larvae of Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) before larval instars complete their development, causes inaccurate estimates of parasitoids impact on CPB, because larvae experience less than their normal lifetime exposure to the parasitoids.

In order to understand the role of introduced parasitoids in pest dynamics, an accurate assessment of parasitoid impact is obviously needed (Waage and Cherry 1992). The evaluation of parasitoids in biological control and the construction of insect life-tables both need the estimation of mortality due to parasitism (van Driesche and Bellows 1988; Bellows *et al.* 1989; van Driesche *et al.* 1991). In other words, a knowledge of the contribution of natural enemies to supression of pests at critical times may be important for classical biological control and conservation and augmentation of parasitoids (Waage 1992). In the effective management of DBM, the successful establishment of specific natural enemies and the maximising of their contribution by manipulation of cropping practices is crucial (Waage and Cherry 1992). Indeed it is only after detailed ecological studies, which identify the specific impact of introduced natural enemies on insect pest populations, that one can evaluate methods for improvement of a biocontrol program (Waage and Mills 1992).

2.3.2. Conventional density estimates and limitations

To estimate losses due to parasitism of an insect species, a number of analytical methods have been presented (Ryan and Medley 1970; Weseloh 1976; Southwood 1978; Torgensen and Ryan 1981; Bellows and Birley 1981; Bellows *et al.* 1982; Manly 1987; van Driesche and Bellows 1988; Bellows *et al.* 1989; van Driesche *et al.* 1989; Bellows *et al.* 1992). Mortality estimates can be provided from density estimates which are typically collected in most population studies (van Driesche *et al.* 1989). Parasitism is calculated by dividing the number of parasitised hosts by the number of hosts collected (van Driesche 1983; Bastian

and Hart 1990; Daigle *et al.* 1990). Percentage parasitism as a measurement of parasitoid activity is a poor indicator of actual parasitoid impact on hosts (van Driesche 1983). This method gives mean percentage parasitism for all sample dates, which may underestimate parasitoid impact in some situations. There are several weaknesses associated with sampling of naturally occurring host populations (Simmonds 1948), which include removal of acceptable hosts from further attack, recent fluctuations in host or parasitoid populations at the time of sampling, variations in duration of a given life stage of the host due to parasitism, variable availability of hosts, multiple attack by parasitoid species, and parasitoid-induced mortality (Petersen 1986; Day 1994) where host mortality is caused by parasitoids but parasitoid progeny are not produced. However, conventional sampling of insect densities is generally considered to provide the best estimate of the host population density and the relative abundance of parasitoid species attacking a naturally distributed host population (Petersen and Watson 1992).

2.3.3. Recruitment method

To evaluate the effect of insect parasitoids on their host populations, two quantities must be measured - the total number of hosts recruited into a susceptible stage, and the number that subsequently become parasitised (van Driesche *et al.* 1991). Because individuals in an insect population are lost through death or alternatively recruitment into the next stage, at no time are all members of the current generation present to be counted (van Driesche *et al.* 1991). To illustrate this, van Driesche and his co-workers (1991) presented the analogy of a partly filled water sink (i.e., the population), into which water is flowing (recruitment) and from which water is draining (death or advancement to the next stage).

Direct measurement of host and parasitoid recruitment is one of the most accurate methods for estimating the impact of parasitoids (Birley 1977; van Driesche 1988a, b; van Driesche and Bellows 1988; Lopez and van Driesche 1989; van Driesche *et al.* 1991). Several other approaches for measurement of recruitment to the host population were discussed by van Driesche *et al.* (1991). A series of models in relation to the host-parasitoid phenological interactions were constructed to evaluate methods for estimation of mortality (van Driesche 1983). These demonstrated the inaccuracy of conventional density estimates and confirmed the validity of the results obtained with the recruitment method. van Driesche *et al.* (1989) presented a method which estimates survival of a given stage from recruitment and density data, a method considered applicable to both continuously breeding insect populations and

insects with discrete generations. Other accurate approaches to the measurement of parasitism are the "short marker stage method" (van Driesche and Bellows 1988; van Driesche 1988a) and the "trap host method" (Ryan and Medley 1970; Weseloh 1976; Torgensen and Ryan 1981; van Driesche and Bellows 1988; van Driesche 1988a; Lopez and van Driesche 1989; van Driesche *et al.* 1991). Waage and Cherry (1992) discussed the importance of using the recruitment and graphical methods in the measurement of parasitism of DBM.

2.3.4. Other methods

Several stage frequency analysis techniques estimate numbers entering a stage or series of stages from counts of densities of the various stages over time (Dempster 1956; Richards *et al.* 1960; Southwood and Jepson 1962; Kiritani and Nakasuji 1967; Manly 1974,1976,1977,1987,1989; Ruesink 1975; Bellows and Birley 1981; Bellows *et al.* 1982). One such analysis is the graphical technique (Southwood and Jepson 1962) where the density of each stage on each sampling date is plotted against time and the total area under the plot is divided by the average development time of that stage in the population. This method is subject to errors due to differential mortality of parasitised and unparasitised hosts between parasitism and parasitoid emergence (Waage and Cherry 1992). Growth-rate analysis methods estimate the mortality by using population growth rates as predictors of population increase between sample dates (Bellows *et al.* 1992). Another method for estimating mortality rates is death rate analysis which estimates total parasitism in insects. Using this method neither stage densities nor recruitment rates can be measured readily, but only mortality rates (Gould *et al.* 1990; van Driesche *et al.* 1991).

There are six experimental methods for evaluation of the overall impact of biological control (Luck *et al.* 1988). These are (1) introduction and augmentation (quantitative evaluation of pest densities both before and after the release of natural enemies), (2) use of cages and barriers (exclusion or inclusion techniques with cages), (3) removal of natural enemies (insecticidal exclusion and hand removal), (4) prey enrichment (addition of prey or hosts to a field), (5) direct observation (sight-count method), and (6) chemical evidence of natural enemy feeding (serological methods, electrophoretic techniques and prey marking used to assess predator-prey interactions).

2.4. Management of diamondback moth

During the past 30 years farmers have relied on insecticides for the control of DBM due largely to their relatively low cost and reliability (Talekar and Shelton 1993). The development of resistance to different classes of insecticides and lack of new ones have led to a search for alternative measures and to the development of IPM (Integrated Pest Management) program (Talekar and Yang 1993). The introduction of parasitoids as an alternative control has been attempted in a few countries such as Malaysia, Indonesia, and the Caribbean islands (Lim 1986).

Conservation, and use of natural enemies, predators, parasites, and pathogens is essential in insect management, because they individually and collectively influence densities (Huffaker and Rabb 1984). Many workers have noted reasons for the failures in biological control (e.g., Hall and Ehler 1979; Hirashima *et al.* 1989; Stiling 1990; Perfect 1992; Hopper and Roush 1993), but insufficient ecological knowledge of target pests and natural enemies is paramount (Ooi 1992). So too is the application of non-selective insecticides to control insect pests which have serious adverse effects on the natural enemies.

An IPM program for DBM may involve a combination of measures such as crop rotation, threshold spraying, destruction of plant residues after harvest (Poelking 1992), physical barriers and toxicants (Endersby and Morgan 1991), the use of selective insecticides least harmful to parasitoids, provision of flowering plants to supply nectar to parasitoids (Waterhouse 1992; Poelking 1992), overhead water sprays (Tabashnik and Mau 1986; Talekar *et al.* 1986; Waterhouse 1992), plant resistance (Waterhouse 1992; Perfect 1992), and the use of trap crops (Talekar *et al.* 1986; Waterhouse 1992; Srinivasan and Krishna Moorthy 1992; Luther *et al.* 1996). In future, inoculative releases of parasitoids, conservation of natural enemies, mating disruption, and cultural controls are a number of tactics that must be considered in areas with acute control problems (Talekar and Shelton 1993). A focus on the use of biological control agents and cultural methods of control, together with little emphasis on the use of pesticides, are sustainable strategies for the management of pests of vegetables (Syed 1992). Additionally, resistance management, including the problem of resistance of insect pests to pesticides of all sorts, is a key component of successful IPM of DBM in the future (Perfect 1992). There are significant

advantages in DBM IPM programs which include natural enemies, partially resistant host plant cultivars, and suitably-applied selective toxicants (Verkerk and Wright 1994).

CHAPTER 3

ASSESSMENT OF PARASITISM IN THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE)

3.1. Introduction

Evaluating the activity of natural enemies in situations where their effect is not so clear is a challenge for biologists (Waage 1992). In order to determine which parasitoids are most effective, an accurate assessment of parasitoid impact is needed (Waage and Cherry 1992). A number of factors have been suggested as causes for the lack of success in biological control. One important factor is the use of adequate techniques to measure parasitoid activity accurately under field conditions and thereby to assess the impact of parasitoids on pests. An essential step in the evaluation of parasitoids in biological control is the measurement of mortality due to parasitism (van Driesche and Bellows 1988). Although there are several weaknesses associated with sampling of naturally occurring host populations (Simmonds 1948), conventional sampling of insect densities is generally considered to provide the best estimate of the host population density and the relative abundance of parasitoid species attacking a naturally distributed host population (see Southwood 1978 for an overview of such methods). However, when pests are collected in the field and subsequently reared, the percentage parasitism is a poor indicator of actual parasitoid impact on hosts (van Driesche 1983).

Considerable effort has been expended in the development of methods to estimate the impact of parasitoids on their host populations (Ryan and Medley 1970; Weseloh 1976; Southwood 1978; Torgensen and Ryan 1981; Bellows and Birley 1981; Manly 1987; Bellows *et al.* 1982; van Driesche and Bellows 1988; Bellows *et al.* 1989; van Driesche *et al.* 1989). One of the most accurate involves measurement of host and parasitoid recruitment (Birley 1977; van Driesche 1988a, b; van Driesche and Bellows 1988; Lopez and van Driesche 1989; van Driesche *et al.* 1991). Another is the direct detection of parasitoid eggs in trap hosts (van Driesche 1983; van Driesche *et al.* 1991).

In this chapter, the seasonal incidence of DBM and levels of naturally occurring larval parasitism were determined on rapeseed *Brassica napus* L. cv. 'Rangi', and kale *Brassica oleracea* var. *acephala* L. cv. 'Grüner' in the Adelaide region between 1993 and 1995. Furthermore, field data on the seasonal prevalence of DBM pupae and pupal parasitism are reported. Several methods were compared as to their suitability to measure the percentage parasitism of DBM by *Diadegma* spp. This study focuses on the role of *D. semiclausum* as a larval parasitoid by relating its temporal association with host to the stage-specific mortality of the host.

3.2. Materials and methods

3.2.1. Study site

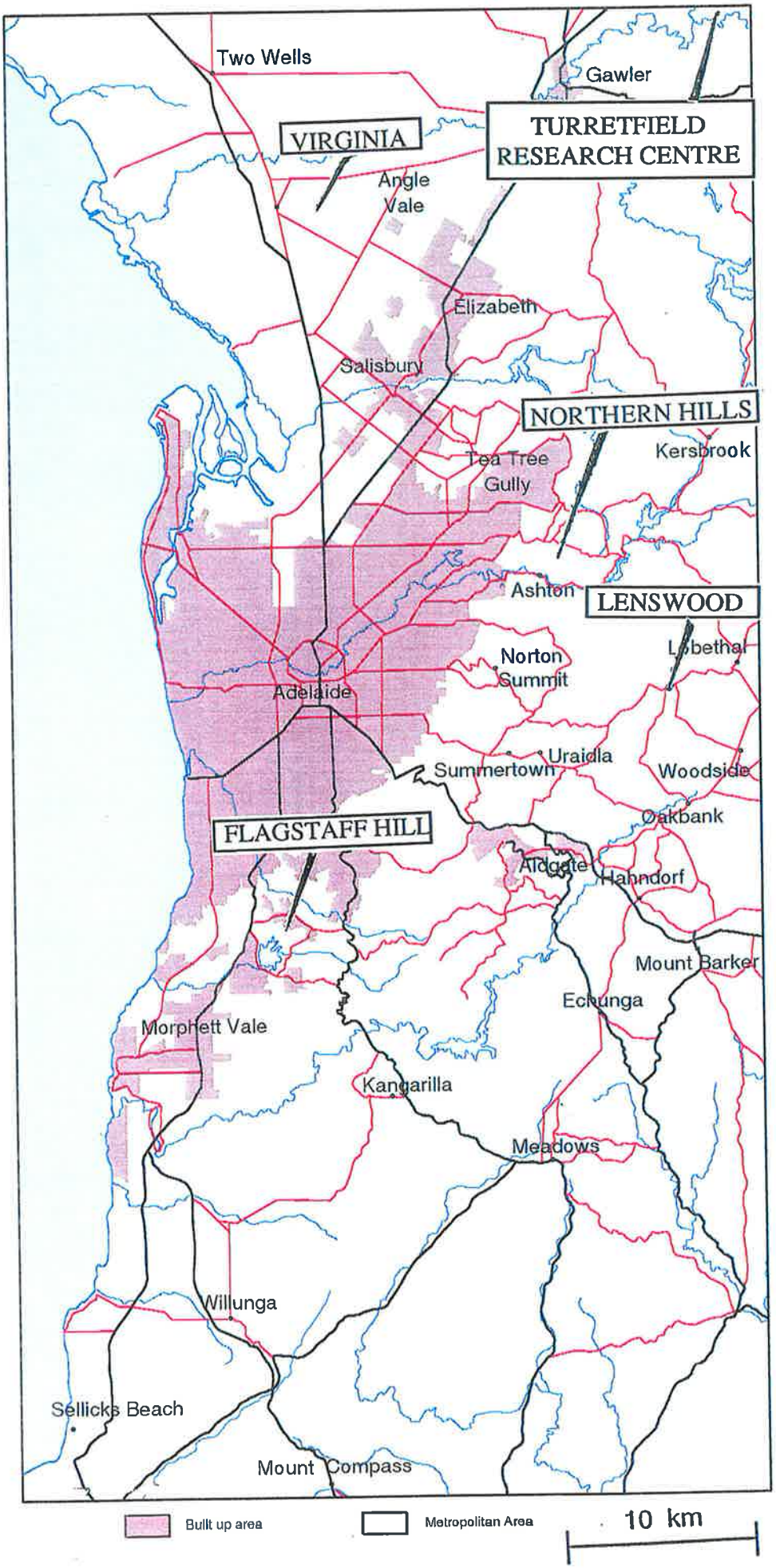
The study was conducted at Lenswood (S 34° 55′, E 138° 52′, elevation 425 m) 20 km east of Adelaide, South Australia (Figure 3.1). To census larval DBM, the site was sampled weekly from 1993-1995. The location was an area of 80 ha, of which approximately 1.58 ha was planted to vegetable research plots and the majority of the remainder to orchard trees. A further 0.2 ha used for this study was divided into three plots: a 0.1 ha rapeseed, and two 0.05 ha plots of kale. Neither the orchards adjacent to field plots nor their surrounds had been sprayed. Weeds including the brassicaceous weed wild radish (*Raphanus raphanistrum* L.) were present.

3.2.2. Plots and planting dates

1000 m^2 of rapeseed was planted on 25 June 1993 and contained about 112,000 plants. Of these, 280-420 were removed for sampling on each occasion which constituted only 0.25-0.4% of the total plot. Similarly a 500 m^2 plot of kale was planted on 15 September 1993 and contained 50,000 plants, of which 250-375 plants (0.5-0.75%) were removed at each sampling. These "early" and "late" plantings provided host plants attractive to DBM throughout the entire season. Again in 1994, 500 m^2 plots of kale were planted on 25 June and 15 September. The individual plot size was chosen such that not more than 10% of the plants would have been destructively sampled by the end of the season. All plots were direct-seeded with a fertiliser spreader at 5 kg/1000 m^2 and seed was incorporated with harrows and rolled with a tyre roller. Natural rainfall provided water, but plots were irrigated by sprinkler in severely dry conditions in December and January when plants were

Figure 3.1. Locations of sampling sites for field surveys of *P. xylostella* and its parasitoids in the Adelaide region.

Adelaide Metropolitan Area



under water stress and the DBM population density was relatively low. No sprays of any kind were applied. Daily maximum and minimum temperatures were obtained from Lenswood Research Centre (Table 3.1).

Table 3.1. Average monthly minimum and maximum temperatures (°C) and rainfall (mm) at Lenswood Research Centre, SA during 1993-1995.

1993

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Av min	14.4	12.9	12.0	11.7	9.4	6.5	6.8	7.7	7.6	7.9	9.9	11.6
Av max	25.3	25.3	22.2	21.3	16.7	11.5	12.1	14.7	15.2	17.3	21.4	22.4
rainfall	44.6	39.6	24.4	3.2	58.0	91.6	118.2	105.2	91.6	90.2	37.2	87.4

1994

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Av min	11.8	12.5	12.0	11.2	8.0	7.5	6.6	5.0	6.6	9.3	9.4	13.1
Av max	22.4	25.0	24.3	20.0	15.5	12.5	13.0	12.0	14.5	18.5	19.3	26
rainfall	83.8	24.6	0.2	27.6	80.6	187.0	65.8	49.2	50.2	106.6	83.0	13.6

1995

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Av min	13.3	14.4	11.3	9.1	7.9	7.2	6.0	7.4	7.1	8.5	11.0	10.7
Av max	26.7	26.5	21.8	16.7	13.7	12.6	9.9	14.5	14.6	17.4	20.9	22.1
rainfall	25.8	31.2	14.6	70.0	152.6	152.4	356.6	57.6	64.4	49.8	28.8	14.2

3.2.3. Data collection

3.2.3.1. *Density sampling methods*

Larvae and pupae from brassicaceous crops were collected by random quadrat sampling. Each quadrat was 50 cm × 50 cm. Weekly sampling commenced after the first pair of true leaves emerged in plots (Table 3.2). On each sampling occasion, 10-15 randomly selected

quadrats of rapeseed or kale plants were destructively sampled by walking through the field and choosing plants according to a random number table and a scheme based on the number of paces along the width and length of plots. Samples were collected from each quadrat as follows. Firstly, without otherwise disturbing the plants, each plant was cut off

Table 3.2. Sampling period of rapeseed and kale plants at Lenswood, SA, 1993-1995.

Crop	First sample	Last sample	No. of samples (quadrats)
Rapeseed	7 Sep. 93	30 Oct. 93	80
Kale	7 Nov. 93	31 Jan. 94	125
Kale	18 Oct. 94*	23 Jan. 95	130

*Samples were taken from early September but no DBM were presented initially.

at 5 cm above the ground and shaken (in groups of 2 to 3 stalks) directly into a plastic sheet funnel (100 cm long, 50 cm wide at the top, 7 cm wide at the base) for ca. 10-15 sec. Larvae were collected in clear plastic cups (7 cm diam. × 8 cm). Each cup was provided with a host plant leaf as food and capped with a perforated plastic lid. A 3 × 3 cm piece of paper towel was placed in each cup to prevent the accumulation of moisture inside, this being a potential source of mortality for larvae during transportation. Larvae were transferred to the laboratory in a portable ice box that also held several ice packs. For collecting the possible remaining larvae, plant samples in each quadrat were placed individually in paper bags, sealed, labelled, and returned to the laboratory. Plant samples were examined further for DBM larvae and/or pupae in the laboratory. For logistical reasons, the larvae collected on each sample date were pooled in containers during rearing.

Sample sizes varied according to the number of larvae per quadrat. One quadrat constituted a sample at a site. Weekly sampling was conducted during the morning hours (7.00-11.30 am) in spring or the late afternoon (4.00-7.30 pm) to avoid the hottest time of the day. Sampling for larvae and/or pupae, to see if there were any, continued through January to mid-February the next year. Population sizes were quantified as the number of larvae or pupae per quadrat, and then converted to numbers of larvae or pupae per m^2.

The total number of samples depends on the degree of precision required (Southwood 1978). All larval instars (except first instar larvae) and pupae were recorded. Sample size was calculated by using Southwood's (1978) formula with $N = S^2 / (X^2 \times E^2)$ in which S = standard deviation, X = mean density, E = accuracy level or the predetermined standard error as a decimal of the mean. Taking the appropriate numbers of samples on each sampling occasion is necessary. However, there were difficulties in the early spring when the DBM infestation was very low. The maximum number of quadrats that could be taken on each sampling occasion was 20 due to time constraints, while when densities were very low over 100 samples generally were necessary for the desired level of precision. Sample sizes were adjusted to attain a 15% precision level throughout the range of the DBM larval densities present or a maximum of 20 samples were taken. It was not possible to calculate variances for % parasitism directly from the data since larvae were pooled during rearing. Therefore, standard errors for mean % parasitism were estimated by assuming that the variable was binomially distributed around the mean (Zar 1984). These estimated standard errors indicate the expected magnitude of variation in samples.

During periods of scarcity, particularly in early spring, it was impossible to collect a representative sample of host larvae. Thus, the first larval generation of 1993 was thought to have been entirely missed, and in 1994 no larvae were collected in the initial six consecutive weekly samples.

3.2.3.2. *Larval rearing*

In the laboratory, DBM larvae were counted and classified as first, second, third and fourth instars to determine the age distribution of the larval population. In general, the first instar was ignored due to its small size and leaf mining habits; very few first instars were collected. Whenever needed, the head width of a larva was measured to the nearest 0.1 mm using a 40 × binocular microscope provided with an ocular micrometer to confirm the instar (Herminanto 1995). In most instances, larvae were classified to instar by inspection under low magnification as this became accurate with experience. Following identification of larval instar, they were placed in groups of 10 in plastic cups (7 cm diam. × 5 cm) with a fresh leaf of host plant for further development. Each cup was capped with a perforated plastic lid and kept at 24±1°C, 14L:10D photoperiod. The room was illuminated by fluorescent lights. Larvae were monitored daily and supplied with leaves of

glasshouse-grown brassicaceous crops until pupation, emergence of a parasitoid and/or DBM adults, or death.

3.2.3.3. *Pupal rearing*

From late November, the pupae of DBM and its parasitoids were collected to assess the incidence of parasitism or possible hyperparasitism. Pupae were placed in plastic cups (7 cm diam. × 5 cm) and kept in a rearing room as described for larvae and allowed to emerge. All pupae that failed to eclose were dissected and examined for parasitoids and hyperparasitoids. Adult parasitoids were identified to species. Determination of *Diadegma* species was based on the venation of forewings as described by Gauld (1984) (Chapter 2).

3.2.3.4. *Estimation of parasitism*

Percentage parasitism was scored in samples collected from 1993 to 1995 by rearing all DBM larvae or pupae collected on each sample occasion. The percentage parasitism and the percentage mortality related to unknown factors were measured from field samples. The relative abundance of the parasitoids was determined from crude estimates of percentage parasitism. Percentage parasitism was calculated by dividing the number of parasitised DBM larvae by the total number of larvae and multiplying by 100. Casualties from causes other than parasitism were omitted from consideration in calculating percentage parasitism. Larvae parasitised by *Diadegma* spp. at earlier instars develop into the 4th instar. In general, the highest rate of parasitised larvae was found in fourth instars on all sample dates (Appendices 1-2) and was therefore taken as an indicator of the rate of parasitism. Mortality was caused by parasitoids of the larva, and other unexplained factors probably including wounding or predisposition to predation and disease. In all instances, parasitism was related to the sampling date and not to the date of adult emergence. Those larvae which died from unknown causes (e.g., disease) were not dissected for presence of parasitoids, because such mortality was assumed not to have been the result of parasitism *per se*.

3.2.3.5. *Trap hosts*

The feasibility of the trap host method for measurement of parasitism was assessed on December 30, 1993 and on January 21, 1994. DBM instars 2, 3, and 4, obtained from a

laboratory culture, were placed on kale plants in the census field to monitor larval parasitism. Larvae were marked individually with Testors® enamel paint which has no detectable effect on behaviour or survival. Marks were quite permanent during the experimental period. All larvae were given at least 30 minutes to resume normal behaviour before they were released. Marked larvae were placed on the leaves of the target plants, and a few of the surrounding plants were removed. In this experiment 90 larvae of each of the last three instars were released onto plants in groups of 15 larvae of one instar only and 6 groups of 5 larvae of each instar together. The larvae were exposed to attack by parasitoids for 24 hr. Locations of the plants on which larvae had been released were marked by placing a brightly colored flag in the ground at the base of the plant for later reference. When larvae were released on kale plants, minimum and maximum temperatures were 8.3°C and 20.7°C in 1993, and 10.0°C and 19.0°C in 1994. Target plants and surrounding plants were searched for marked larvae on the following day. After recovery, the larvae were placed in plastic cups (7 cm diam. × 5 cm) and kept at 24±1°C until parasitoids or adult moths emerged.

3.2.3.6. *Recruitment method*

In seeking to quantify the impact of parasitoids, it was decided to assess parasitism with a recruitment method hitherto not attempted in previous studies on parasitoid impact on DBM populations. Based on the method proposed by Lopez *et al.* (1993), DBM cocoons were sampled to estimate host and parasitoid recruitment per quadrat per sampling interval and percentage parasitism by the parasitoids. Recruitment of DBM cocoons was scored by examining all plants in 10 to 21 quadrats. The number of quadrats was determined using data from the preceding sampling occasion. Quadrat sites were selected randomly. All cocoons (but not larvae) were removed and quadrats were marked with brightly colored flags. 3-4 days later all DBM or parasitoid cocoons found on the plants in marked quadrats were counted and taken to the laboratory where they were placed in plastic cups (7 cm diam. × 5 cm) and kept at 24±1°C to be reared. A series of such samples taken over the season could be summed to estimate total recruitment. Upon emergence, the parasitoid adults as well as DBM adults abandoned their cocoons and left them on the host plant. Pupal cases from which adult moths or parasitoids had emerged also were counted. Because there is no movement of the cocoons between plants, such cocoons found on previously stripped plants were considered as recruits that have entered the pupal stage.

The number of DBM cocoons containing parasitoids was used to estimate parasitoid recruitment. New quadrats for recruitment estimation were selected for each sampling occasion. The number of cocoons recruited and total losses to parasitism were calculated in each interval. As done with density samples, standard errors for % parasitism were estimated by assuming that the variable was binomially distributed (Zar 1984).

DBM usually pupates on the host plant (Harcourt 1957), but in this study, when the density was very high (late November and early December) they often pupated on weeds amongst kale plants. For example, many cocoons were collected from knotweed, *Polygonum aviculare* L. (Polygonaceae), nettle, *Urtica urens* L. (Urticaceae), and lambsquarters, *Chenopodium album* L. (Chenopodiaceae). Therefore, weeds as well as crops were sampled within quadrats.

3.3. Results

3.3.1. Seasonal abundance of *P. xylostella*

All instars were found on the foliage of kale plants on all sample dates throughout the spring and mid summer of both years. The presence of all instars throughout this period indicates that DBM has overlapping generations. There was a distinct peak on 30 November 1993 and another on 6 December 1994 in larval numbers attributable to large numbers of 4th instars (Figures 3.2-3.3; 3.4a-3.5a). In the kale plots parasitism by *D. semiclausum* gradually increased during December 1993 and there were distinct peaks of DBM parasitism in mid January 1994 (Figure 3.4b), and mid November 1994 and early January 1995 (Figure 3.5b). The density of DBM larvae varied from being almost absent up to as many as 348 larvae per m^2 (Figures 3.4a-3.5a).

Larval density of DBM gradually increased from mid spring, peaked in late November and rapidly decreased thereafter (Figures 3.4a-3.5a). A rapid collapse of the larval population in the December period coincided with the onset of hot weather and peak parasitism in early January (Figure 3.4a). Later (mid January-August 1994), DBM all but disappeared in both cultivated fields and on weeds (Chapter 4). Larval parasitism by *D. semiclausum*, too, dropped from a minor peak of 9% on 7 November to 3.5% on 23 November and thereafter began to rise in early December with a consequential decrease of DBM larval density. High

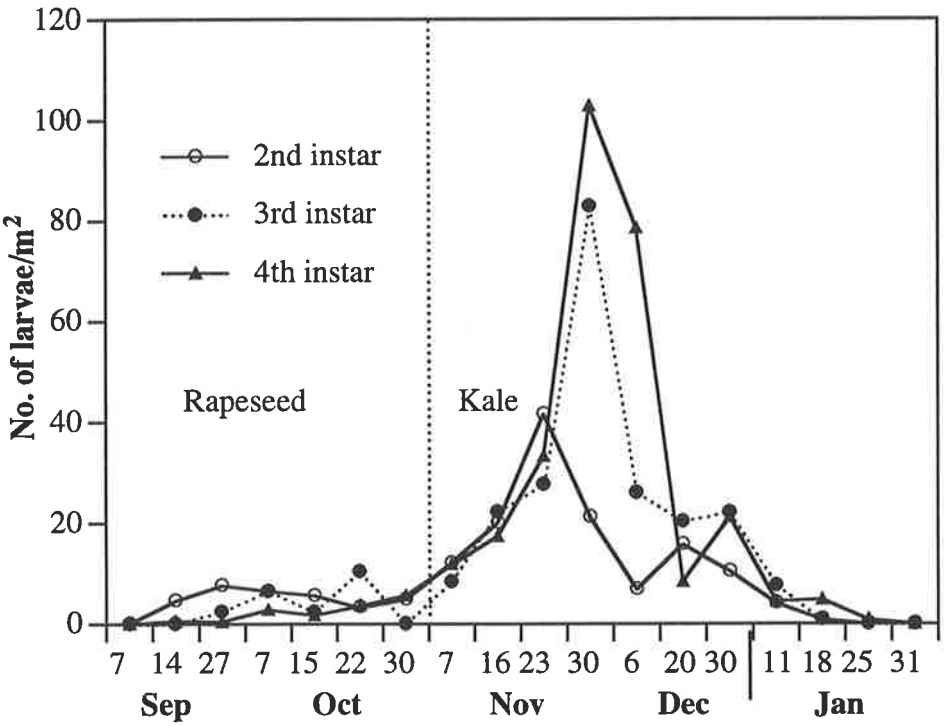


Figure 3.2. Age structure of larval *P. xylostella* from rapeseed and kale at Lenswood, South Australia 1993-1994.

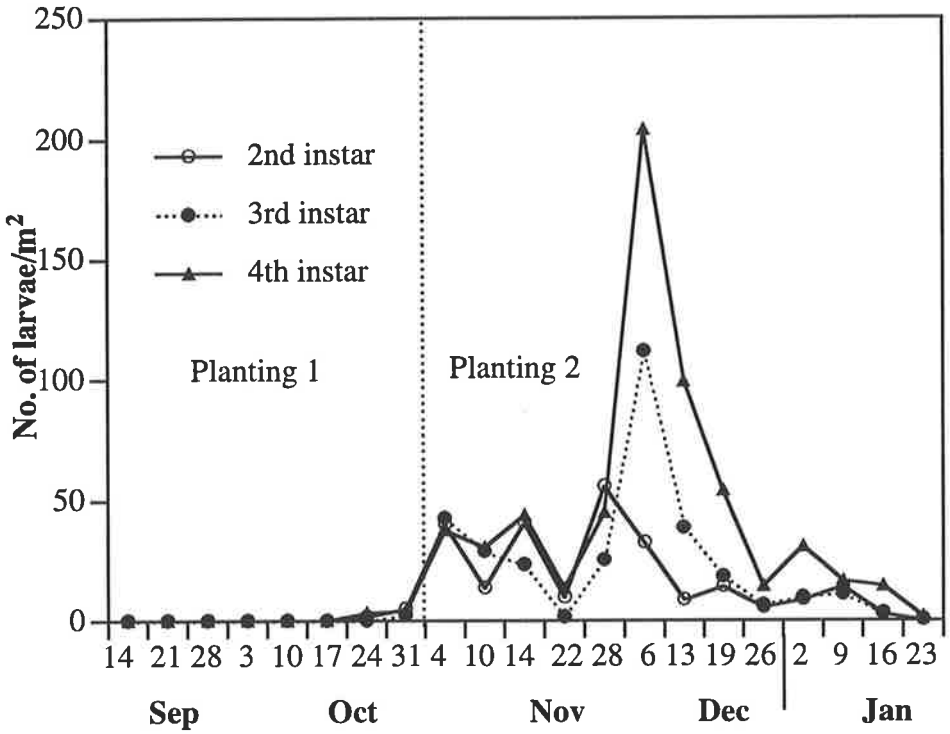


Figure 3.3. Age structure of larval *P. xylostella* from kale at Lenswood, South Australia 1994-1995.

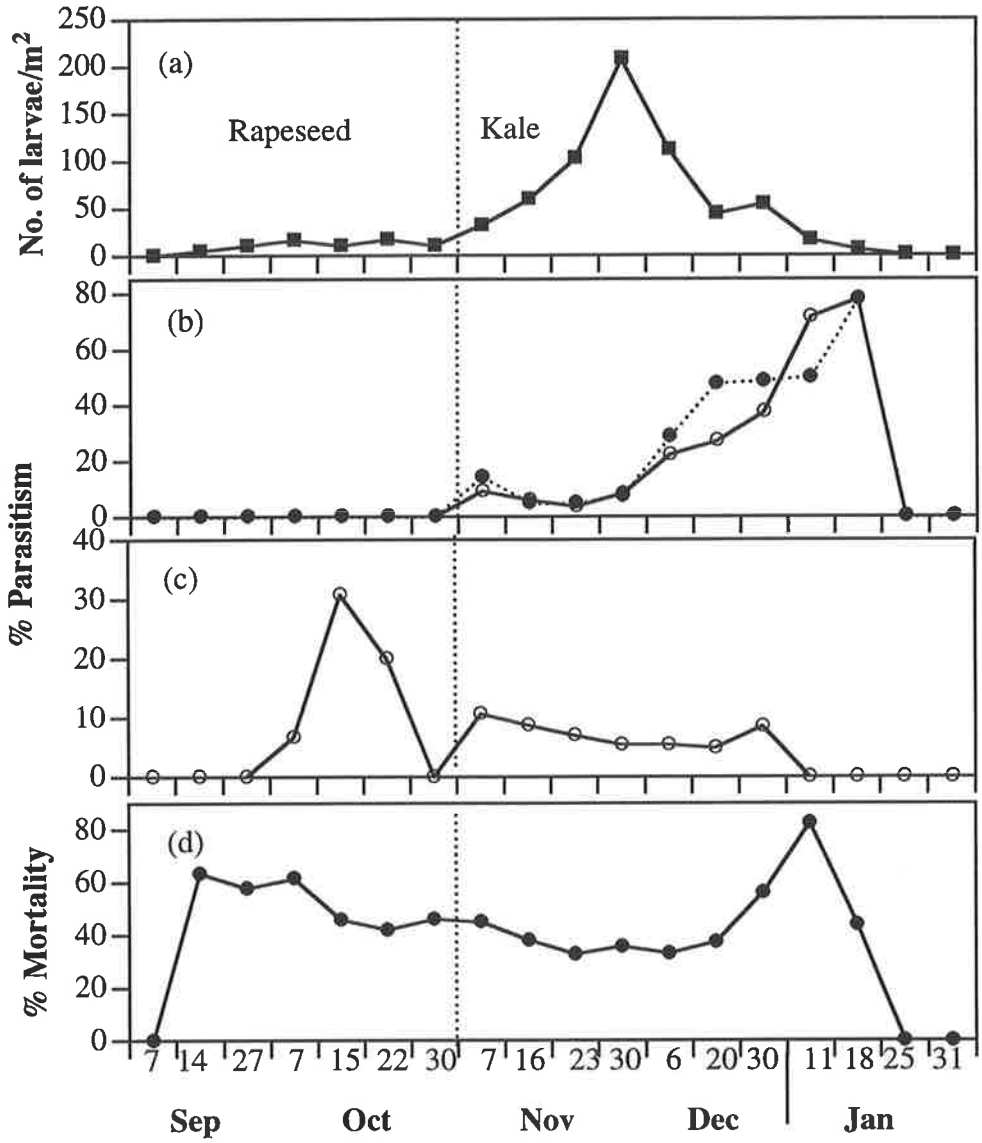


Figure.3.4. Larval density, percent parasitism and unexplained mortality of DBM based on the conventional density sampling method on rapeseed and kale at Lenswood, South Australia 1993-1994; (a) larval density of DBM; (b) percent parasitism attributed to *D. semiclausum* of three last instars (open circles) and 4th instar only (closed circles); (c) percent parasitism by *A. ippeus* ; and (d) unexplained mortality.

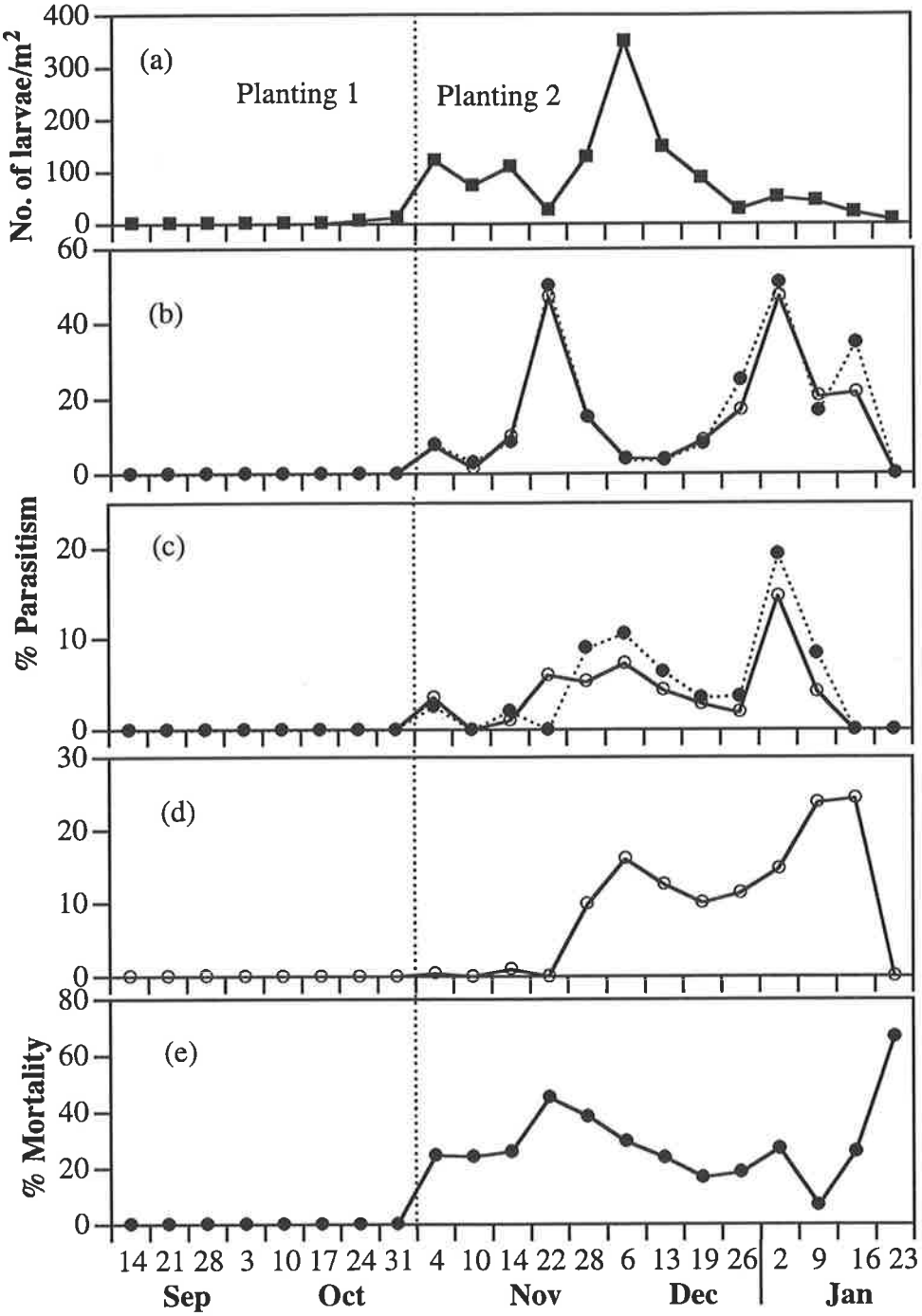


Figure 3.5. Larval density, percent parasitism and unexplained mortality of DBM based on the conventional density sampling method on kale at Lenswood, South Australia 1994-1995; (a) larval density of DBM; (b) percent parasitism attributed to *D. semiclausum* of three last instars (open circles) and 4th instar only (closed circles); (c) percent parasitism attributed to *D. rapi* of three last instars (open circles) and 4th instar only (closed circles); (d) percent parasitism by *A. ippeus*; and (e) unexplained mortality.

parasitism was then maintained until mid January (Figure 3.4b). In 1994, larval parasitism was low up to mid November, then peaked on 22 November and also on 2 January in 1995, when larval density was low (Figure 3.5b).

3.3.2. Mortality factors affecting DBM larvae and pupae

A total of six species of hymenopterous parasitoids, including three larval and two pupal parasitoids, were reared from DBM in 1993-1995 (Table 3.3). An unknown species of eulophid, parasitised a single larva during the study.

Table 3.3. Parasitoids reared from DBM larvae and pupae sampled from rapeseed and kale at Lenswood, SA 1993-1995.

Species	Family	Stages parasitised
Diadegma semiclausum Hellen	Ichneumonidae	Larva
Diadegma rapi (Cameron)	Ichneumonidae	Larva
Apanteles ippeus Nixon	Braconidae	Larva
Unknown sp*	Eulophidae	Larva
Diadromus collaris (Gravely)	Ichneumonidae	Pupa
Brachymeria phya (Walker)	Chalcidae	Pupa

* one specimen.

Of the 1154 DBM larvae that were reared during the 1993-1994 growing seasons (Table 3.4), 150 were parasitised by *D. semiclausum* and 79 by *Apanteles ippeus* Nixon (parasitism of 13.0% and 6.8%, respectively), with a combined total larval parasitism of 19.8%. In 1994-1995 when 1980 larvae were reared, 196 were parasitised by *D. semiclausum*, 99 by *Diadegma rapi* (Cameron), and 223 by *A. ippeus* (parasitism of 9.9%, 5.0%, and 11.3%, respectively), with a combined total larval parasitism of 26.2%. The average total larval parasitism in 1993-94 was somewhat lower than 1994-95. This increase between the 1993-94 and 1994-95 growing seasons is mostly attributable to an increase in the numbers of *D. rapi* and *A. ippeus* (Table 3.4). Results for *D. semiclausum* parasitism of 2nd, 3rd, and 4th instars showed high percent parasitism of the 4th instar in the field (Appendices 1-2). The eggs of *Diadegma* spp. hatch inside the host, and their

Table 3.4. Total parasitism of DBM by the larval parasitoids *D. semiclausum*, *A. ippeus* and *D. rapi*, and the pupal parasitoids, *B. phya* and *D. collaris* as determined from conventional density samples taken from rapeseed and kale at Lenswood, SA 1993-1995.

	1993-1994	1994-1995
Total larvae collected	1916	2700
Total larvae died*	762	720
Total surviving larvae	1154	1980
Total *D. semiclausum*	150	196
Total *A. ippeus*	79	223
Total *D. rapi*	0	99
Total larval % parasitism	19.8	26.2
Total pupae collected	339	575
Total pupae died*	0	64
Total surviving pupae	339	511
Total *B. phya*	75	116
Total *D. collaris*	55	29
Total pupal % parasitism	38.3	28.4

*Unexplained mortality.

larvae remain undeveloped in it. They accumulate in 4th instar larvae, and then develop and pupate inside the host cocoons. Furthermore, the fourth instar *per se* is prone to parasitism but to a lesser degree than earlier instars (Chapter 6). Fourth instars under field conditions develop slower and remain in the field longer than second and third instars. By remaining longer in the field, parasitised and unparasitised fourth instars were usually collected more often in samples. Thus, parasitised 4th instars approximate the total parasitism by *Diadegma* spp. accumulated over all four instars. There was no substantial difference in rates of parasitism calculated from larvae overall and the rate in 4th instars alone (Figures 3.4-3.5). This is not surprising as the majority of larvae in samples were 4th instars.

In 1993, of the 339 pupae collected, 130 or 38.3% included pupal parasitoids, while in 1994, of the 511 pupae, 145 or 28.4% contained pupal parasitoids (Table 3.4). DBM pupal density was greatest each year from late November through December and then decreased (Figure 3.6). In general, *B. phya* and *D. collaris* were first observed around the end of November. There were two peaks of activity for *B. phya*, one in late December and another in mid-January. The seasonal pattern of activity of *D. collaris* differed between years. In 1993 there was a single period of parasitism by *D. collaris* with a peak in mid of December, whereas in 1994 it was active throughout December and January.

3.3.3. Unexplained larval mortality

In 1993-94, percentage mortality caused by unexplained factors peaked at 63.2% on 14 September and was 82.5% on 11 January (Figure 3.4d). Similarly in 1994-95, percentage mortality peaked at 45% on 22 November and was 66.7% on 23rd January (Figure 3.5e). Relatively large numbers of diseased larvae were seen in the field when unexplained mortality was high.

3.3.4. Trap host method

In the trap host experiments, 29.7% and 31.1% of the total released larvae were recollected in 1993 and 1994, respectively. Most larvae were lost. DBM larvae tended to move from the plant on which they were placed. Movement to a new position or a new plant occurred for all instars. Fourth instars were recaptured more frequently than the other instars. In 1993 and 1994, 18 and 21 of the total fourth instar larvae recaptured in each year were

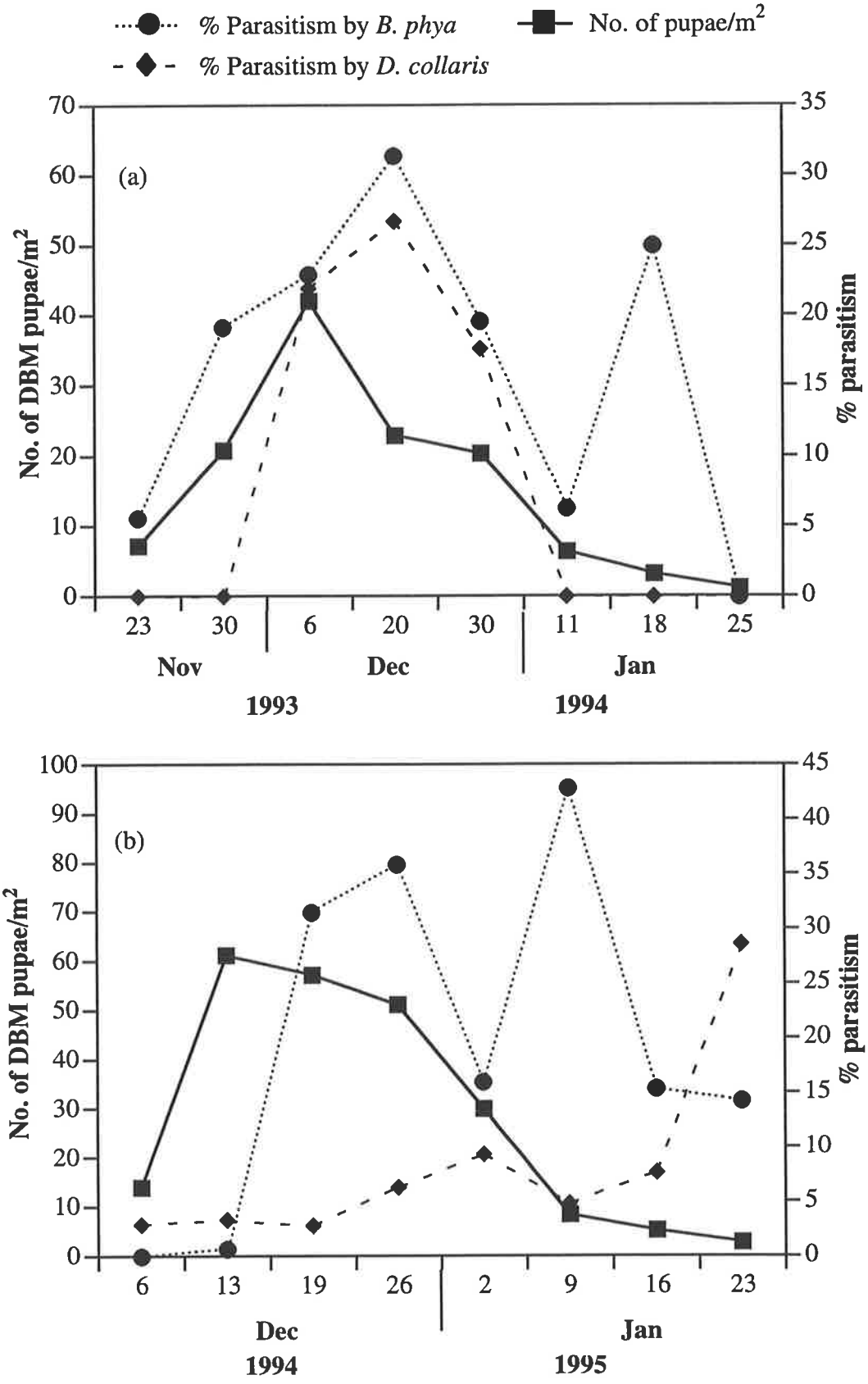


Figure 3.6. Seasonal change in number of *P. xylostella* pupating per m^2, and percentage parasitism due to *B. phya*, and *D. collaris* at a kale field at Lenswood in 1993-1994 (a), and 1994-1995 (b) respectively.

found on neighbouring non-target kale plants, respectively. Fourth instars were recaptured 24 hr after release mostly at various locations on the marked plants and on leaves and branches of other plants that overlapped the marked ones. Because less than 50% of marked larvae were recaptured, the data were not statistically analysed (Table 3.5). One of the reasons that most larvae were not recaptured was related to their dispersal; the larvae moved readily from target plants to others. Rearing recaptured 2nd, 3rd, and 4th instars indicated that each of these instars were parasitised by

Table 3.5. Recapture statistics for marked DBM larvae released on kale plants at Lenswood, SA.

a) December 1993

Instars	No. larvae marked	No. larvae recaptured	% larvae recaptured	Range	Mean±SE /plant	No. larvae parasitised
2nd-alone	90	16	17.8	0-5	2.7±0.71	2
3rd-alone	90	26	28.9	2-7	4.3±0.76	4
4th-alone	90	36	40.0	3-8	6.0±0.85	2
2nd-mixed	30	5	16.7	0-3	0.8±0.47	1
3rd-mixed	30	11	36.7	1-3	1.8±0.30	1
4th-mixed	30	13	43.3	1-3	2.2±0.30	1

b) 1994

Instars	No. larvae marked	No. larvae recaptured	% larvae recaptured	Range	Mean±SE /plant	No. larvae parasitised
2nd-alone	90	15	16.7	0-6	2.5±0.84	3
3rd-alone	90	36	40.0	4-9	6.0±0.85	5
4th-alone	90	34	37.8	2-12	5.7±1.56	2
2nd-mixed	30	5	16.7	0-2	0.8±0.40	0
3rd-mixed	30	7	23.3	0-3	1.2±0.47	1
4th-mixed	30	15	50.0	1-4	2.5±0.42	1

D. semiclausum in the field and between these, more parasitoids emerged from second and third instars than from fourth instars.

The results of these experiments indicated that the parasitoid is able to parasitise the 4th instars in the field albeit at a low frequency. As recapture rates were so low, the trap host approach was considered to be inappropriate for measurement of DBM larval parasitism by *D. semiclausum* in the field.

In summary, the results of this experiment showed that although 2nd-4th instars were naturally parasitised after 24 hr, estimating DBM larval parasitism by *D semiclausum* and/or *D. rapi* in the field conditions based on this approach was not accurate. However, only *D. semiclausum* emerged from recovered larvae. *D. rapi* and other parasitoid species were not recorded.

3.3.5. Recruitment of DBM and its parasitoids

In kale plots, recruitment of DBM cocoons began on 10 November 1994 and was low until early December. Then substantially peaked from 16 to 19 December at 55 cocoons/m^2/day (Figure 3.7a).

Parasitism by *D. semiclausum* from 22% on 18 November dropped to 2% on 1 December then reached again 23% on 6 December and was not so high until early January. Thereafter it began to rise in mid-January and peaked at 46% when pupa density decreased (Figure 3.7a). *D. semiclausum* recruitment peaked on 19 December at 5.5 cocoons/m^2/day. This was coincided with the peak of DBM, however, percent parasitism was low (about 10%). Whereas, the rate of parasitism by *D. rapi* peaked on 6 December at 17% when pupal recruitment was low at 3 cocoons/m^2/day then dropped to 0% on 16 December while DBM recruited at 21cocoons/m^2/day (Figure 3.7b). *D. rapi* recruitment peaked on 13 December at 2 cocoons/m^2/day, this was coincided with DBM recruitment at 23 cocoons/m^2/day and rate of parasitism was 7%. However, the rate of parasitism ranged from 0% to 4.5% during November.

Total losses due to parasitism by *D. semiclausum* estimated from host and parasitoid recruitment into pupal stage was 17.25% and 25.55% in November and January

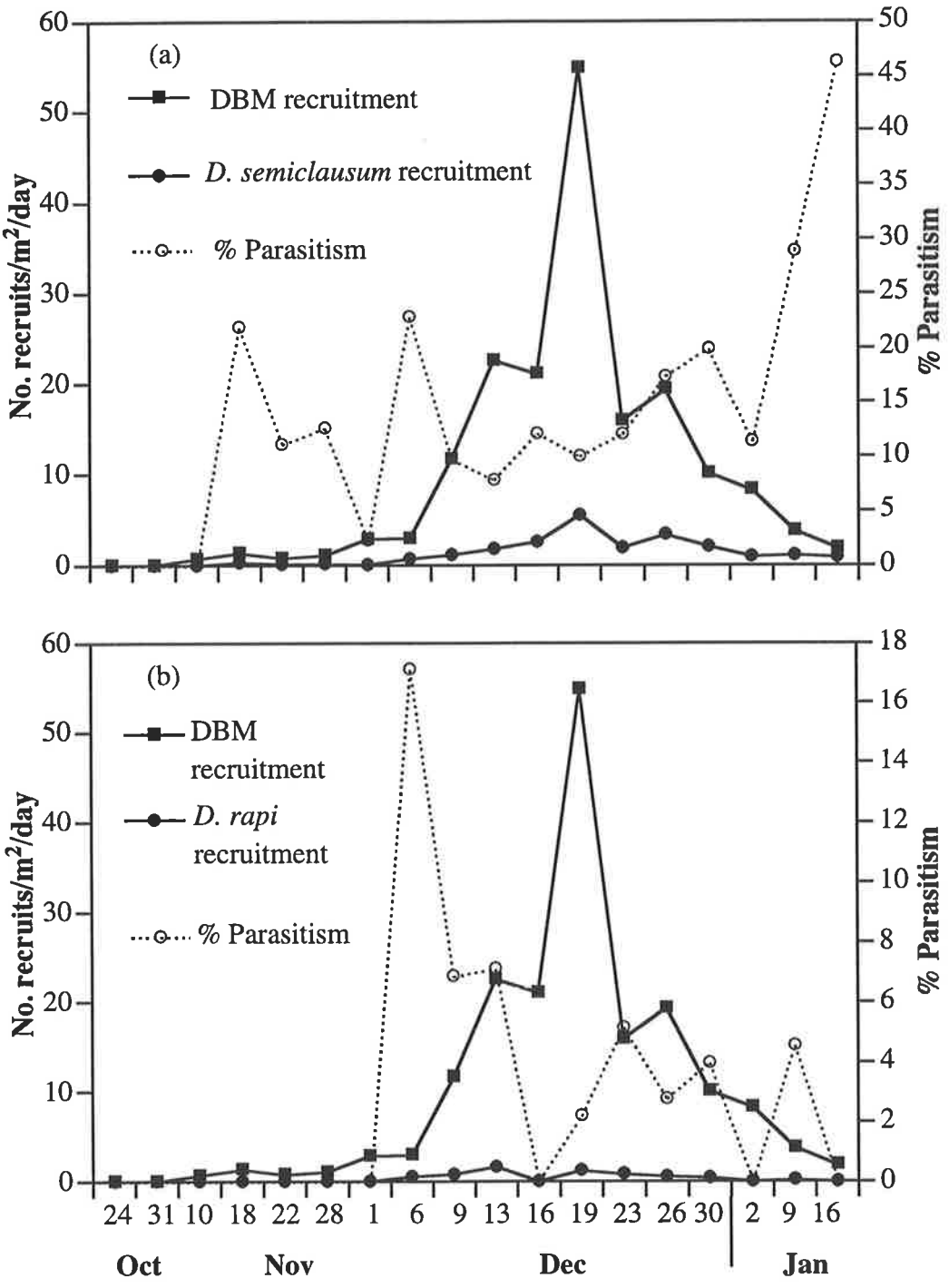


Figure 3.7. Daily recruitment of pupal DBM and its parasitoids on kale at Lenswood, South Australia 1994-1995; (a) by *Diadegma semiclausum* and (b) by *D. rapi* .

respectively. This increase could have arisen in different ways: (i) greater parasitoid than host recruitment during January, (ii) earlier recruitment of parasitised than healthy hosts into the pupal stage. Explanation (ii) is not tenable because parasitoids affect the development of their hosts, thus a developing parasitoid may keep a host in one stage long after healthy hosts have moved to a later stage (Waage and Cherry 1992). Therefore explanation (i) based on the proportion of host and parasitoid recruitment (this proportions were 6 and 4 times in November and January respectively) is a possible explanation of the seasonal (mid January) increase in percentage parasitism. Percent parasitism from density (conventional) samples was somewhat lower than parasitism calculated by the recruitment method.

3.4. Discussion

Three methods were utilised to assess parasitism of DBM by *Diadegma* spp. One of these was the conventional density sampling method whereby larvae and/or pupae were collected and reared until DBM adults or parasitoids emerged. Because of this, total parasitism of *Diadegma* spp. accumulated over all four instars was measured. The second method, the recruitment method, involved the collection of DBM cocoons where percent parasitism was calculated directly from adult emergence. The other, the trap host method, was based on releasing 2nd to 4th instars onto host plants, then recapturing and rearing them to estimate percent parasitism.

It was considered likely that estimates of % parasitism based on conventional density estimates would be biased. However, it might be possible to reduce such a bias by focusing on parasitism of 4th instars in view of the cumulative nature of parasitism at the 4th instar. Laboratory experiments (Chapter 6) showed that although *Diadegma semiclausum* oviposits directly into all instars, the second and third instars are preferred and there is little parasitism of 4th instars (Lloyd 1940; Velasco 1982).

Three major difficulties were encountered in using DBM 4th larval instar parasitism as an index of DBM parasitism. The numbers of 4th instars collected in September and January were low and usually inadequate (Figures 3.2-3.3). The second difficulty was the high larval mortality rates under natural conditions (unexplained mortality) (Figures 3.4d-3.5e).

Thus, some parasitism could not be followed to the 4th instar. In other words, if the mortality of parasitised larvae during development differed from mortality of unparasitised larvae, then this would introduce a bias into the estimates of % parasitism. Furthermore, removal of larvae at the time of sampling prevented their exposure to further parasitism. 4th instars parasitised by *Diadegma* spp. remain in the field longer, and are therefore more available than unparasitised larvae, and thus would be over-represented in samples. This third factor leads also to overestimation of percentage parasitism for 4th larval instar (Waage and Cherry 1992).

Consider a hypothetical example (Table 3.6). Assume that the proportion of total developmental time spent in each stage (1st to 4th instar) is approximately equal for parasitised and unparasitised larvae, and that all stages are present in equal density. If one focuses on the 4th instars, then approximately half of the development of the 4th instar will be completed at the time of sampling while all development in the earlier instars is completed. From the analysis in Table 3.6, one would conclude that percent parasitism of 4th instars by *D. semiclausum* would underestimate total parasitism by approximately less than 10%. As mortality is cumulative, from any given cohort, the fraction of first instars that survives would be greatest and the fraction of 4th instars that survives will be least. Therefore, underestimation of parasitism should be even less, given that fewer of the 4th instars will typically be available for parasitism. But parasitised 4th instars take longer to develop than unparasitised 4th instars which would tend to produce an overestimate in % parasitism. These factors tend to cancel each other and the final extent of bias in the estimated % parasitism is unknown. Temporal variation in oviposition and mortality will also affect the accuracy of such an estimate. However, because total parasitism is accumulated over all four instars, measurement of parasitism of 4th instars is likely to provide a better indication of parasitism by *Diadegma* spp. than estimates based on rearing all larval instars. It can be used as an indicator of the relative abundance of the individual *Diadegma* species within the complex present at a particular location. However, one must always be aware that there are unknown biases in such estimates.

Sampling in the field will rarely recover 100% of the intended organisms. Thus, it was expected that some minor underestimation of DBM density would occur. As mortality is cumulative, one would expect that, over long periods of time, the greatest numbers of

insects in a sample would come from the younger stages. This expectation must be modified to take the duration of development into account. If one stage lasts longer than another; then the longer stage will occur relatively more frequently in samples. Thus, given the difference in developmental times between the 3rd and 4th instars of DBM, one would expect that there would be at most 1.3 times as many 4th instars as there are 3rd instars in

Table 3.6. Larval DBM developmental period and percent parasitism at constant temperature.

Instar	Duration of development at 20°C (days)*	Fraction of stage completed when 4th instars are sampled	Susceptibility to parasitism/day**	Overall susceptibility to parasitism	Expected fraction of parasitism during lifetime
1	4.5	1	0.48	2.16	0.30
2	2.8	1	0.66	1.84	0.25
3	3.4	1	0.56	1.90	0.26
4	4.5	0.5	0.32	1.44	0.20

*: from Salinas (1986); **: from figure 6.2 (Chapter 6).

samples when data are pooled over long periods of time. Inspection of Figures 3.2 and 3.3 suggests that sampling of 2nd and 3rd instars was less efficient than sampling of 4th instars. The extent of this bias appears to be small given the different developmental times of these instars (Table 3.6), but the overall level of bias is unknown.

Overall, many larvae were lost when the trap host method was used. Moreover, the presence of two *Diadegma* species in the field prevents estimation of the rate of parasitism by any one species through dissection. Therefore, the recaptured larvae must be reared, during which some of these larvae may die by unknown factors that cause an inaccurate estimate of parasitism. The performance of this method is variable. For instance, for sessile host stages the performance is generally good, but for mobile hosts, host behaviours can create problems if trap hosts fail to act "naturally" (van Driesche *et al.* 1991). For example, Gould 1990 (cited in van Driesche *et al.* 1991) indicated that the trap host larvae

did not accurately measure the rate of parasitism in the gypsy moth. Although the mobility of larval instars in DBM system caused most of the larvae placed on the target plants to fail to be recovered, parasitism in the larvae which were recaptured indicated that all the last three larval instars (2nd, 3rd, and 4th instar) were parasitised by *D. semiclausum* in the field. Between these instars, more parasitoids emerged from second and third instars than the 4th instar in a given interval. It should be noted that when DBM is attacked, it usually drops from the host plant. Such larvae may move to other plants. Thus, the trap host method would probably underestimate the incidence of parasitism.

Knowledge of the number of individuals entering the susceptible host stage and those which afterward become parasitised is required for the accurate assessment of parasitism (van Driesche and Bellows 1988). This can be accurately estimated by scoring the recruitment of host and parasitoid individuals, which provide a direct estimate of the numbers of individuals entering the relevant stages (van Driesche and Bellows 1988). DBM can be parasitised in all instars by *Diadegma* spp. It would not be possible to measure recruitment into each instar as well as recruitment of parasitoids according to instar. Small larvae, especially 1st instars, are very difficult to sample accurately. Furthermore, it would be extremely difficult to accurately distinguish the different instars in the field. Therefore, it was considered that because all *Diadegma* spp. emerge from pupa of DBM, measurement of parasitism with estimates of recruitment at the pupal stage would be accurate (Figure 3.8). Lopez and van Driesche were confronted by a similar problem with parasitism of Colorado potato beetle; they also used recruitment into the pupal stage to estimate % parasitism. However, DBM larvae may die from other causes and therefore will not be recruited to the pupal stage. If parasitised and unparasitised larvae suffer differential mortality prior to pupation, then estimated % parasitism will be biased. Parasitism as measured by such a recruitment method at the pupal stage will lag that from density samples of larvae, since development of larvae causes a delay.

A delay between peak density of larvae and peak recruitment of pupae was observed (Figure 3.9c). However, the observed delay cannot be explained by delayed development alone. When peak densities occurred in the field, mean temperatures were approximately 20°C (Table 3.1), so the expected delay would be on the order of 2-3 days (1/2 the developmental time of 4th instars; Table 3.6). Therefore, it appears that larval mortality

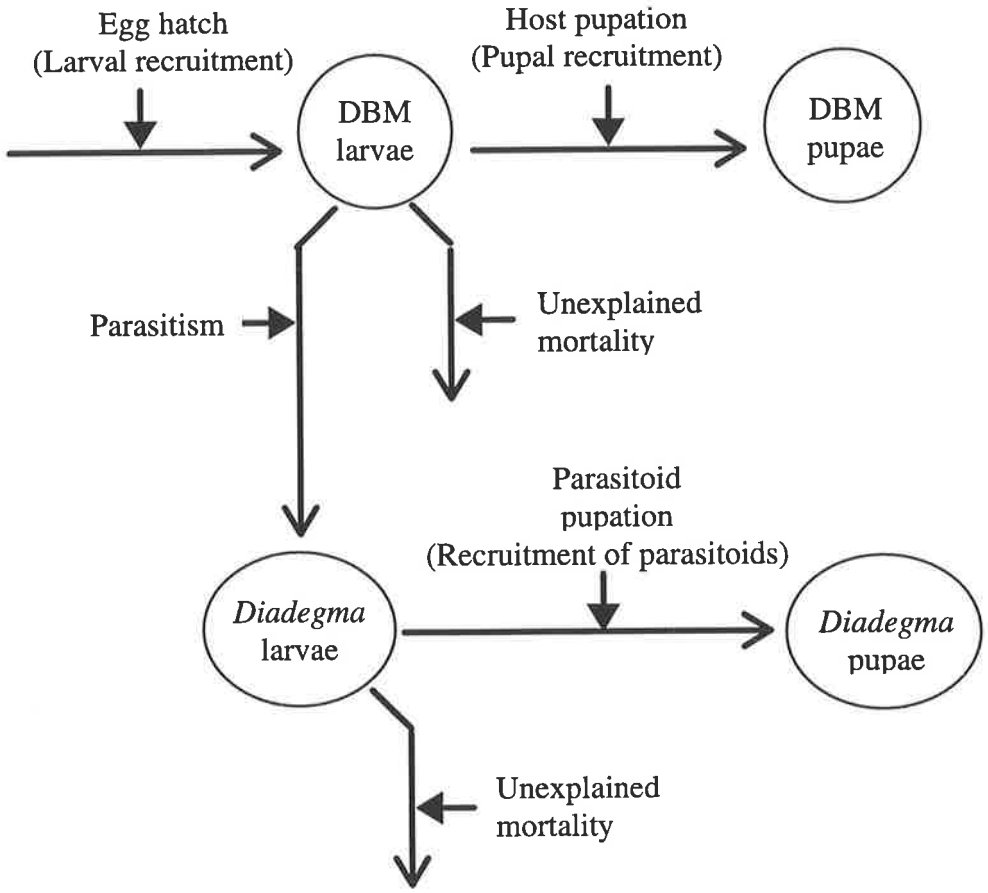


Figure 3.8. Schematic linkages between DBM and parasitic *Diadegma* species.

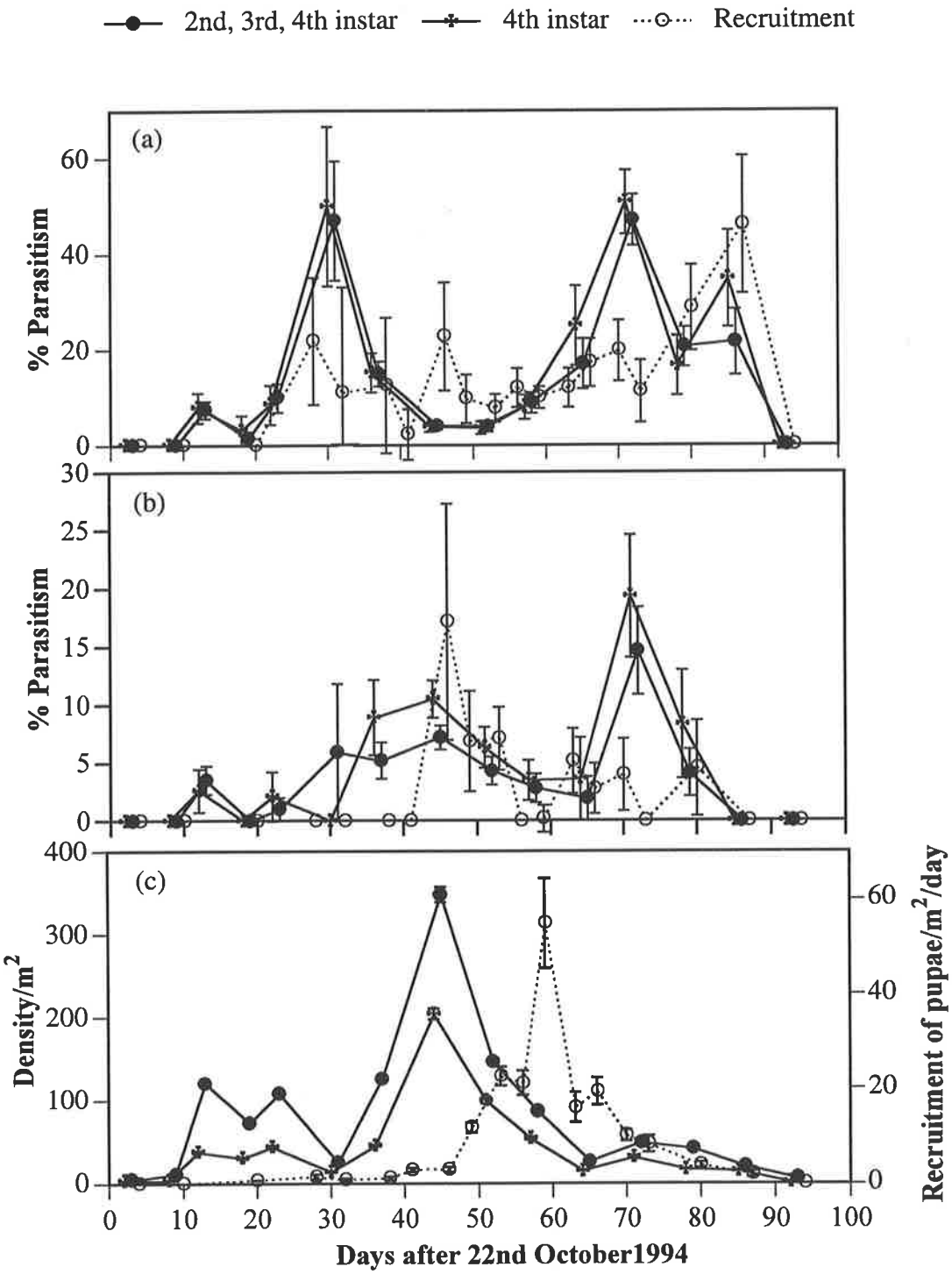


Figure 3.9. Percent parasitism of DBM based on the conventional density sampling and recruitment methods on kale at Lenswood, South Australia 1994-1995. Conventional density estimates were used to calculate larval parasitism on instars 2, 3, 4 together and instar 4 alone. (a) percent parasitism by *D. semiclausum* ; (b) percent parasitism by *D. rapi*; and (c) DBM larval density calculated by conventional sampling and recruitment of pupae. Points for recruitment were shifted one day forward and for instar 4 one day backward to distinguish means and error bars. Error bars indicate standard error of mean. Reference dates: 10 = 1st November; 41 = 1st December; 72 = 1st January 1995.

varied over time and influenced the delay between peaks. There were several minor peaks of larval abundance that were not mirrored in pupal recruitment which reinforces the conclusion that differential mortality occurred over time. These observations indicate that there are probably biases of unknown magnitude in estimates of % parasitism as estimated by the recruitment method. It was concluded that the recruitment method provided an accurate estimate of *ultimate* percent parasitism by *Diadegma* spp., but neglected other associated forms of mortality such as wounding or predisposition to predation and disease.

There are two additional problems with the recruitment method. The acquisition of sufficient cocoon samples for accurate estimates of parasitism can be virtually impossible at times, particularly in early spring. Another problem was that the detection of parasitised hosts among those cocoons that are dead when collected is impossible.

All methods for estimation of % parasitism have limitations and no single method will give accurate estimates of losses due to parasitoids. If densities of DBM do not change very quickly, it is expected that percent parasitism estimates by different methods would be correlated. However, for both *Diadegma* species, the estimated percentage parasitism of DBM measured using the conventional method was not correlated with estimates using the recruitment method (Figures 3.10-3.11). Nor were there correlations between estimates using the 4th instar alone and those using the recruitment method. There was however, for both species, a positive correlation between estimates of percentage parasitism using the conventional samples of instars 2,3, and 4 and 4th instars alone. The correlation between these two methods is expected, since much of the same data were used in calculating both estimates. The lack of correlation between % parasitism calculated by density samples and recruitment methods makes interpretation of seasonal patterns of parasitism difficult. Both methods have biases and it appears that these biases are not the same for both methods. The research focused on *Diadegma* species because these were known to be the most abundant parasitoids of DBM (e.g., Waterhouse 1992; Mustata 1992). It is expected that estimates of parasitism by other species were subject to the same or similar sampling biases as those for *Diadegma*. Therefore, the data can be considered to give only an impression of seasonal patterns of parasitism.

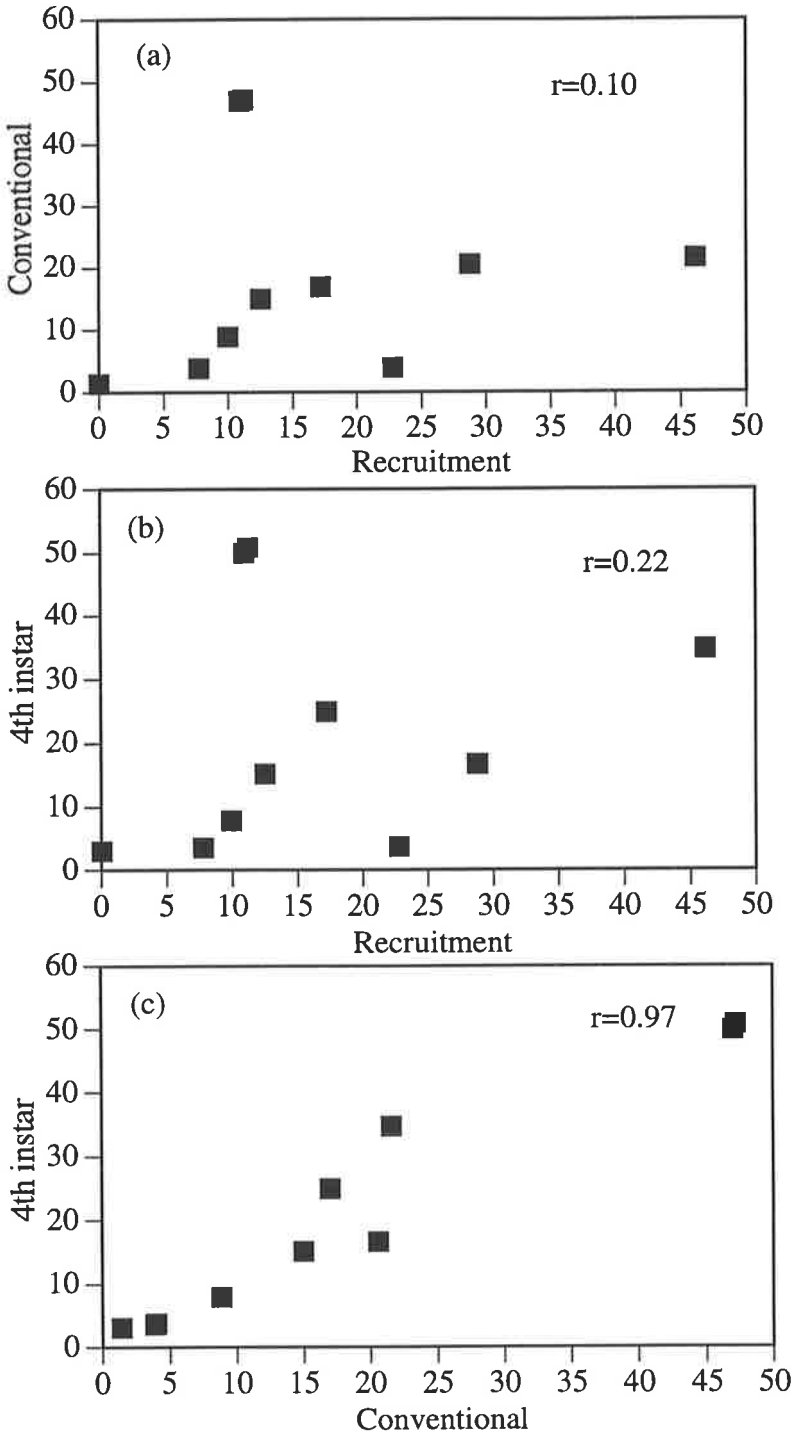


Figure 3.10. Correlations between percent parasitism of DBM by *D. semiclausum* as estimated by three different methods: conventional density sampling, recruitment, and 4th larval instars.

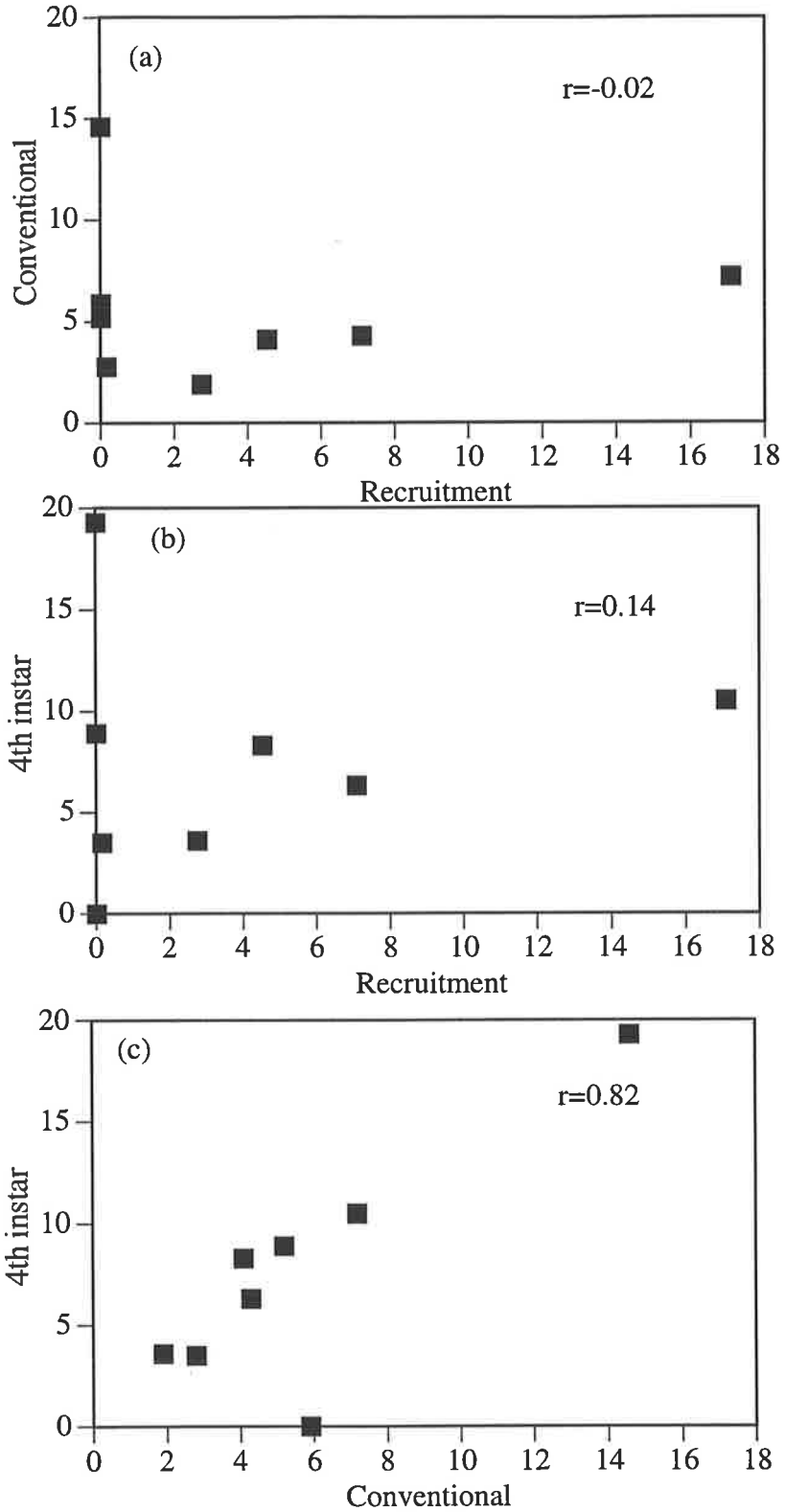


Figure 3.11. Correlations between percent parasitism of DBM by *D. rapi* as estimated by three different methods: conventional density sampling, recruitment, and 4th larval instars.

There are numerous studies which describe the % parasitism of DBM (e.g., Vos 1953; Sastrosiswojo and Sastrodihardjo 1986; Chua and Ooi 1986; Talekar and Shelton 1993). Since most reports of levels of parasitism are based on density samples, they are subject to the same kinds of bias as were found in the study reported here and therefore do not always adequately reflect the effects of parasitoids on DBM. Nonetheless, parasitoids often kill large numbers of DBM. Therefore, it is important to develop further methods to obtain reliable estimates of the % parasitism in DBM.

The density of DBM larva was very low early in the season and increased rapidly in the late spring and early summer generations. Population densities of DBM, however, varied considerably during the study period. Usually, but not always, reductions in larval abundance coincided with increases in percentage parasitism. The temporal decrease in larval density was probably caused by a combination of parasitism, unexplained mortality factors such as disease, and the switching of moth oviposition from older maturing plants to younger kale or other brassicaceous plants in adjacent areas (Chapter 5). In 1993 there was an apparent increase in the rate of parasitism in the early summer. This coincided with a sharp decline in densities of DBM, and hence percent parasitism was subject to estimation errors at this time. The density of DBM larvae in 1993 peaked in late November and in early December 1994 (cf. Figures 3.4a-3.5a). Larval density dropped later in the growing season. This decline may have been caused by the combination of parasitism, host plant senescence and high temperature. Wakisaka *et al.* (1992) reported that survival rates of DBM in the early and mid summer are extremely low and in autumn become high.

The level of unexplained mortality was very different from month to month, and was lower during spring than summer in 1993 and vice versa in 1994 (Figures 3.4d-3.5e). Efforts made to determine the cause of the unexplained mortality were unsuccessful. Second only to this, parasitoids were the major cause of DBM larval mortality in the field. Total larval mortality in DBM was higher in 1993 than 1994 (Appendix 3). Parasitism was observed to vary widely from month to month and even from year to year. This may be a function of the population density of DBM and/or fluctuations in weather conditions. Talekar and Yang (1991) demonstrated that parasitism by *D. semiclausum* (=*eucerophaga*) was low at low (10°C) and high (35°C) temperatures. Parasitism, however, was high in the temperature range of 15°C to 25°C. In this study, and consistent with the findings of

Talekar and Yang (1991), maximum levels of parasitism were observed in November when the average temperature was 14.4°C. Parasitism due to *D. semiclausum* was evident from early November and increased to mid January. There was a distinct lag of peak parasitism following peak larval densities of DBM. Goodwin (1979) indicated a distinct lag only during winter. This lag was attributed to low temperature.

In both years of this study, parasitism by *D. semiclausum* was recorded from 3-6 weeks after the first DBM larvae were found in the field. For example, in 1993 larvae found on 14 September to 30 October escaped parasitism, but those found thereafter were affected (Figure 3.4b). Similarly, in 1994 DBM larvae were initially detected on 24 October and parasitised larvae were recovered on the first rearing of 4 November (Figure 3.5b). Higher rates of parasitism (>20%) were maintained during December. Investigation of the results for all three larval parasitoids showed fluctuations of percent parasitism between the 1993 and 1994 spring and summer seasons. The rate of larval parasitism by *D. semiclausum* varied from 3.5% to 77.8%, and from 1.4% to 47.2% throughout the growing seasons in 1993-94 and 1994-95, respectively. The incidence of parasitism was not positively correlated with DBM density, an observation consistent with many other parasitoid species (Knipling 1992).

D. semiclausum parasitised DBM larvae in both years, while parasitism by *D. rapi* in 1994, and that of *A. ippeus* in 1993, was minor. The predominant parasitoid in 1993-94 was *D. semiclausum* and it was *A. ippeus* in 1994-95. Note that *D. rapi* was absent in 1993-94. During this study the proportion of larvae parasitised and identified as male and female of *D. semiclausum* upon emergence was not significantly different ($\chi^2 = 0.43$, $p = 0.51$, 71 males and 79 females for 1993; $\chi^2 = 0.74$, $p = 0.39$, 92 males and 104 females for 1994). Hyperparasitoids were not abundant; only three unidentified specimens were collected, but there may be greater hyperparasitism of parasitoid pupae which were not sampled.

In 1993 to 1995, parasitism was generally low in the beginning of spring and then increased throughout the sampling period (Appendices 4-5). The results of this study showed that in cultivated brassicaceous crops, *D. semiclausum* and *A. ippeus* are the dominant parasitoids of DBM in South Australia. In 1993-94 parasitism attributable to *D. semiclausum* comprised 41.8% of total parasitism and in 1994-95, 29.6%. This is lower than 82%

reported by Goodwin (1979) for *D. semiclausum* (=*cerophaga*) from Victoria. There were several notable differences between the present and other studies in relation to geography, numbers of parasitoids recaptured, and sampling methods used to estimate percent parasitism. Although, the level of parasitism of *D. semiclausum* and *D. rapi* in the present study was not as high as that reported elsewhere, the role of each of these parasitoids, as a factor attributable to the mortality of DBM, is considerable.

In general, an increase in host pupae density was observed from early December. In 1993 and 1994, host pupae peaked in early and mid December, respectively (Figure 3.6). Thus, host pupal population level fluctuated widely, so that pupal resources for *B. phya* and *D. collaris* were temporally discontinuous at field. Furthermore, because wild brassicaceous habitats of DBM, particularly wild radish *Raphanus raphanistrum* L. and wild mustards *Sisymbrium* spp. were distributed patchily in the Adelaide region at many urban and suburban areas, and also because these host plants developed during spring and were available only by late November each year (Chapter 4), host pupal resources for these two pupal parasitoids were spatially discontinuous. Pupal parasitism was sampled only on crop plants.

The pupal parasitoids attacked the host from late November as long as the pupae were available. From early December onwards, a major proportion of DBM pupae were parasitised by *B. phya* and *D. collaris*. A high level of parasitism was attained in late December in both years (Figure 3.6). They emerge only after DBM damage has occurred in the current generation but may influence populations of DBM in subsequent generations. The pupal parasitism was also observed after mid-January when DBM larval parasitoid activities greatly decline. DBM pupae declined to low densities, and resulted in an overly high estimate of percentage parasitism of DBM pupae. The summer emergence of pupal parasitoids seem to be in temporal asynchrony, rendering these less effective than *D. semiclausum* as biocontrol agents of DBM in brassicaceous farms.

CHAPTER 4

SEASONAL OCCURRENCE OF *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE) AND ITS PARASITOIDS ON BRASSICACEOUS WEEDS IN THE ADELAIDE REGION

4.1. Introduction

DBM feeds on members of the family Brassicaceae including cultivated crops and weeds (e.g., Harcourt 1986; Talekar and Shelton 1993; Muhamad *et al.* 1994). It has also been reported to attack several species from other plant families (Waterhouse and Norris 1987). Wild radish *Raphanus raphanistrum* L., Indian hedge mustard *Sisymbrium orientale* L., hedge mustard *Sisymbrium officinale* (L.), and giant mustard *Rapistrum rugosum* (L.), grow as annual weeds in South Australia (Jessop and Tolken 1986). These weeds may be a source of damaging infestations in crops when they senesce at the end of spring.

The importance of the weedy host plants as an early season habitat, on one hand, and the extent of infestations of DBM on these weeds and their relevance to population dynamics of DBM on crops, on the other hand, were unknown prior to this research. Understanding the ecology of DBM in local cropping systems is a prerequisite for improving management of this pest in crops. Despite this, little is known of the ecology and population dynamics of DBM and its major parasitoid *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) on weedy host plants in South Australia. A study was started in the winter of 1993 to determine population dynamics of DBM and its parasitoids on brassicaceous weeds.

This chapter deals with the seasonal occurrence and abundance of DBM and its larval parasitoids in the Adelaide region of SA (Figure 3.1). The aim was to determine the impact of parasitoids, especially *D. semiclausum*, on DBM larval populations on brassicaceous weeds in areas not sprayed with insecticide, and to study the temporal distributions of these populations. The phenology of wild radish and wild mustards (Appendix 6) in horticultural and non-horticultural areas was investigated in relation to DBM abundance on these plants. The study also focused on identification of other larval parasitoids.

The following specific questions are addressed in this chapter:

1 - How do DBM densities vary on two major wild host plants, wild radish and wild mustards?.

2 - What is the incidence of parasitism on the weeds?

4.2. Materials and methods

4.2.1. Study sites

The survey described in this chapter was conducted at four study sites. Two sites, were in the Adelaide hills region, one at Lenswood in the east and one in the northern Adelaide Hills (S 34° 45′, E 138° 50′) to the north-east of Adelaide. The other two sites were in the plains region to the north of Adelaide, one at Virginia (S 34° 40′, E 138° 33′), a horticultural cropping area and one at Turretfield (S 34° 33′, E 138° 49′), a pasture area (Figure 3.1). Brassicaceous crops were grown in the vicinity of all sites except Turretfield. The sites ranged in elevation from 12-14 m at Virginia to 425 m at Lenswood. The longest distance between sites was approximately 40 km between Turretfield and Lenswood in the hills. The major weedy host plant at Lenswood was wild radish. The landscape of the northern Adelaide Hills, 15 km NE of Adelaide, is a vegetable producing area comprising a mosaic of small sparse brassicaceous vegetable fields and uncultivated scrub areas. In spring much of this area is covered with wild mustards, including hedge mustard, Indian hedge mustard, and giant mustard. At Virginia, cabbage, cauliflower and broccoli are some of the main crops and they are planted sequentially from early spring to early summer. Furthermore, this area was also planted to a variety of vegetables, especially potatoes and onions. In the brassica fields of this region, economically damaging infestations by DBM were observed and some pesticides were applied once per week, and at times of peak infestation two to three times per week. Brassicaceous weeds, including wild radish and wild mustards, grow along road sides and peripheral to cabbage farms (Figure 4.1). At Turretfield, a pasture area, the dominant weed species was the mustard *Sisymbrium orientale* L. which was patchily distributed in the area.

DBM larvae were censused weekly on weeds at Turretfield, Lenswood, and the northern Adelaide Hills during spring, and at the two latter sites monitoring continued in autumn and winter, whereas Virginia was usually visited twice per month until weeds senesced in late spring.

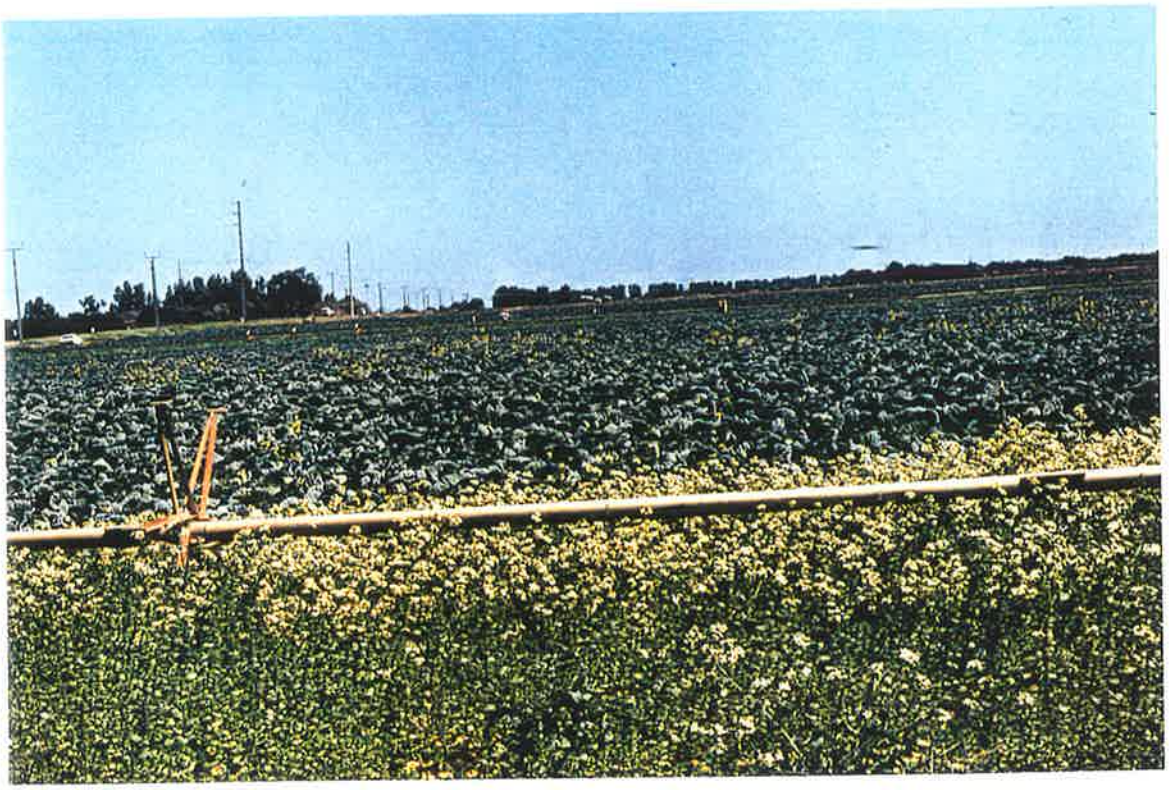


Figure 4.1. Wild radish stands growing peripheral to and amongst cultivated cabbage at Virginia, a vegetable production area to the north of Adelaide, SA.

4.2.2. Collection of larvae by quadrat

In spring1993, samples of larvae from patches of brassicaceous wild host plants, mainly wild radish and wild mustards, were collected using the methods described for collecting larvae by quadrat from brassicaceous crops (see Chapter 3 for detailed methods). Brassicaceous weeds were first visually examined for eggs and feeding damage. Sampling commenced in early September when the flying activities of DBM adults were observed, eggs were found on weedy host plants and larvae or their damage was first noted. Prior to this, DBM populations were too small to be detected on weeds. Sampling continued until late November when weeds senesced. On each sampling occasion, 5-10 randomly selected 50 cm x 50 cm quadrats of weeds were destructively sampled by walking through a transect distance of 10 to 20 m. DBM larval density on brassicaceous weeds in spring was low in 1993, consequently sufficient numbers of DBM larvae to provide estimates of parasitism on weeds were not collected. Larval densities were quantified as the number of larvae per quadrat, and then converted to larvae per m^2.

4.2.3. Collection of larvae by sweep net

In 1994, a sweep net was used for sampling relative larval densities of DBM. This was due to the difficulties of sampling by the quadrat method. When plants had attained a large size, samples were difficult to collect from weeds with quadrats. Also larger numbers of insects were collected with a sweep net, which allowed determination of levels of parasitism. Samples were collected from brassicaceous weeds at Lenswood and in the northern Adelaide Hills at approximately one-week intervals, and twice per month at Virginia. The Turretfield location was not sampled in 1994. All locations were monitored from 10 May when weeds emerged to the end of the third week of November when the weeds senesced. However, larvae were mainly collected during spring from early September until late November as DBM density was too low to readily detect before September. This 12 week period covered most of the activity of DBM larvae and their related parasitoids on the weeds.

A muslin sweep net, 30-cm in diameter, with a 120 cm handle and a 80 cm deep conical bag was used to sample DBM. One sample each at Lenswood and the northern Adelaide Hills consisted of 100 180° sweeps were taken while walking along a 10 to 40 m transect, and one sample at Virginia of 30 sweeps was taken along a 10 to 20 m transect on each sampling date. The numbers of samples differed among sites because weeds were not as

abundant at Virginia. Samples were replicated 3 times (300 or 90 sweeps) with a distance of 5-10 m between transects. Plants were swept as much as possible from the ground up to the top of the plants throughout each location. Larvae in each sample were removed from the sweep net and placed separately into clear plastic cups (7 cm diam. × 8 cm) with a perforated lid.

4.2.4. Larval rearing

In the laboratory, all DBM larvae, were counted and reared using the methods that were described for larvae collected from brassicaceous crops (Chapter 3) to determine the age distribution of the larval population and levels of parasitism by *D. semiclausum* and other parasitoids. Larvae were monitored daily and supplied with leaves of field-collected weeds in 1993, and glasshouse-grown brassicaceous weeds in 1994 until pupation, emergence of a parasitoid and/or DBM adults, or death. Percentage parasitism was calculated with respect to the emerged individuals as was described in Chapter 3.

Fourth larval instar parasitism

To determine the rate of parasitism, larvae collected on each sampling date were separately reared (2nd, 3rd, and 4th instar). Generally, the highest rate of parasitism by *Diadegma* was found in 4th instars. As 4th instars are less susceptible to parasitism, they were used as an indicator of the rate of parasitism by *Diadegma* species.

4.2.5. Parasitoid identification

All the emerged adult parasitoids of DBM larvae were identified to species. Determination of *Didegma* species was based on the venation of forewings, as described by Gauld (1984) (Chapter 2). Three species were collected from weeds: *D. semiclausum, D. rapi*, and *A. ippeus.*

4.3. Results

4.3.1. Seasonal abundance of *Plutella xylostella*

1993

DBM larvae were present on wild radish and wild mustards, in greatest numbers from September to late November. DBM was first collected in quadrat samples on the 7th of September from wild radish at Lenswood and on the 17th of September from wild

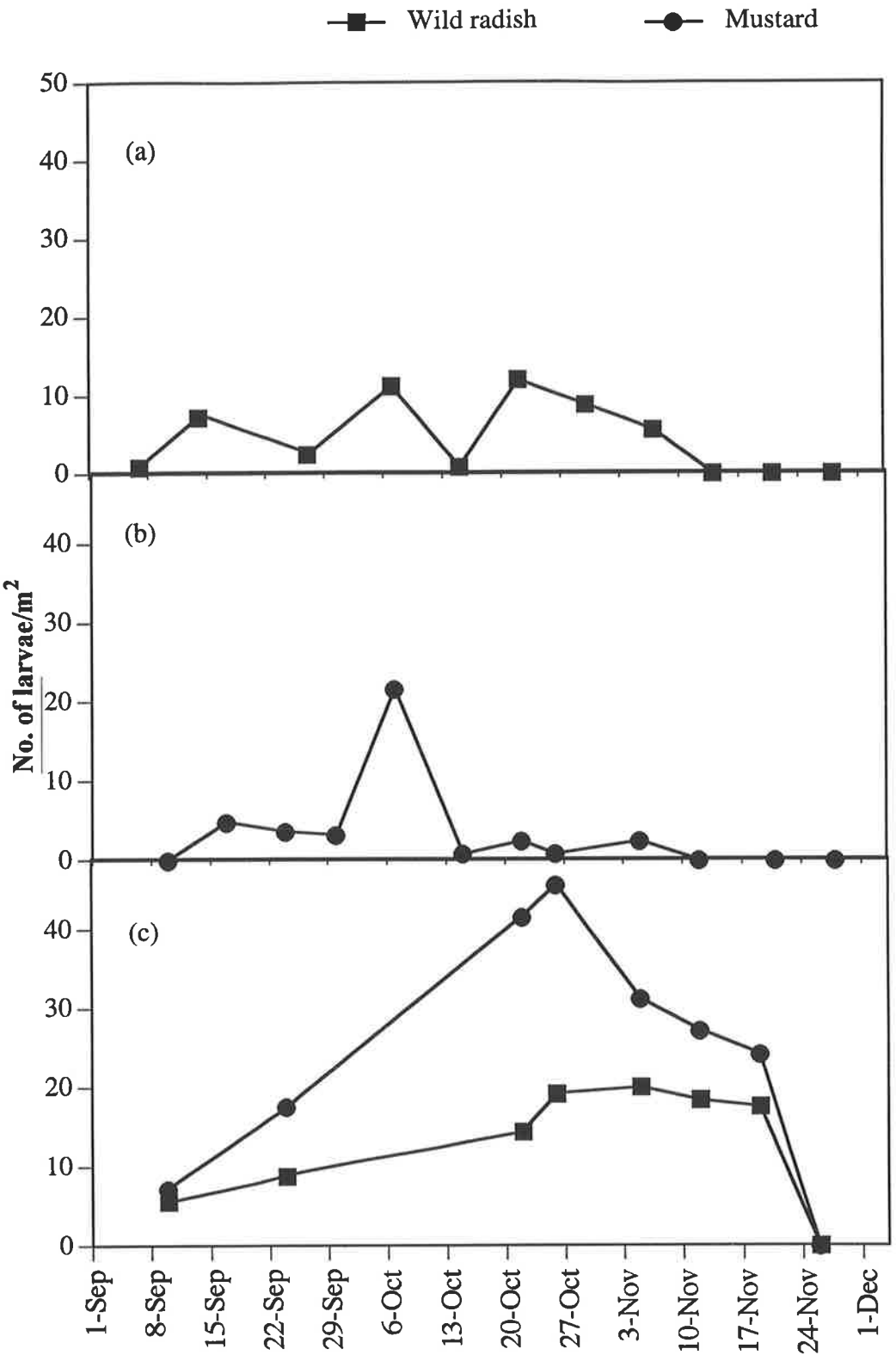


Figure 4.2. Densities of 2nd to 4th instar larval DBM from (a) wild radish at Lenswood; (b) wild mustard at Turretfield; (c) wild radish and wild mustard at Virginia, 1993.

mustards when they were flowering at Turretfield (Figure 4.2a-b). Larvae were collected from the 10th of September at Virginia (Figure 4.2c). The density of DBM larvae was low on wild radish at Lenswood during 1993 and peaked at 12 larvae/m^2 on the 22nd of October (Figure 4.2a). Similarly, the low larval density of DBM on wild mustards at Turretfield during 1993 peaked at 21.6 larvae/m^2 on the 7th of October (Figure 4.2b). At Virginia density peaks of 19.2 larvae/m^2 on wild radish and 45.6 larvae/m^2 on mustards, occurred on 26th of October (Figure 4.2c). Note that the seasonal pattern of occurrence differed among these sites.

1994

In 1994, larvae were first collected from mustards on the 10th of September at both Virginia (Figure 4.3a) and the northern Adelaide Hills (Figure 4.3b) and from wild radish on the 27th of September at Lenswood (Figure 4.3c). In 1994, patches of wild radish among or around the cultivated areas of brassicaceous crops at Virginia were considered too small and sparse to sample DBM. When wild radish and mustards were fully flowering in early October 1994, DBM adults were observed flying and mating on these weeds and on crop plants (Chapter 3). However, it was not until mid October that large numbers of eggs were laid on both the ventral and dorsal surfaces of host plant leaves. This coincided with suspected immigration of DBM into the Adelaide region (Chapter 5). Eggs were laid singly or in groups of up to 4 on surfaces of wild radish and wild mustards leaves and were placed mostly in leaf axils or near the midrib. Most eggs were laid on the less mature leaves in the top two thirds of the plants.

At Lenswood, in 1994, larval densities from early spring (September) to mid October were low on wild radish. The number of larvae per 100 sweeps was 1 by the 10th of October and mean larval densities rose to no higher than 70.7 larvae per 100 sweeps on the 10th of November. Meanwhile, percentage parasitism by *D. semiclausum* reached a maximum of 1.9% on the 24th of October (Figure 4.4a). Larval densities on wild radish at this site declined thereafter. The 28th of November was the last sampling date in 1994, when wild radish plants had matured and were poorly suited for larval feeding or adult egg laying. *D. semiclausum*, *D. rapi* and *A. ippeus* were collected at Lenswood. Of the 4th instars collected from wild radish plants at Lenswood, the overall rate of parasitism by *D. semiclausum* was 1.4% (Appendix 7a). Monitoring on mustards in the northern Adelaide Hills indicated low densities of DBM in early September. The larval population peaked at

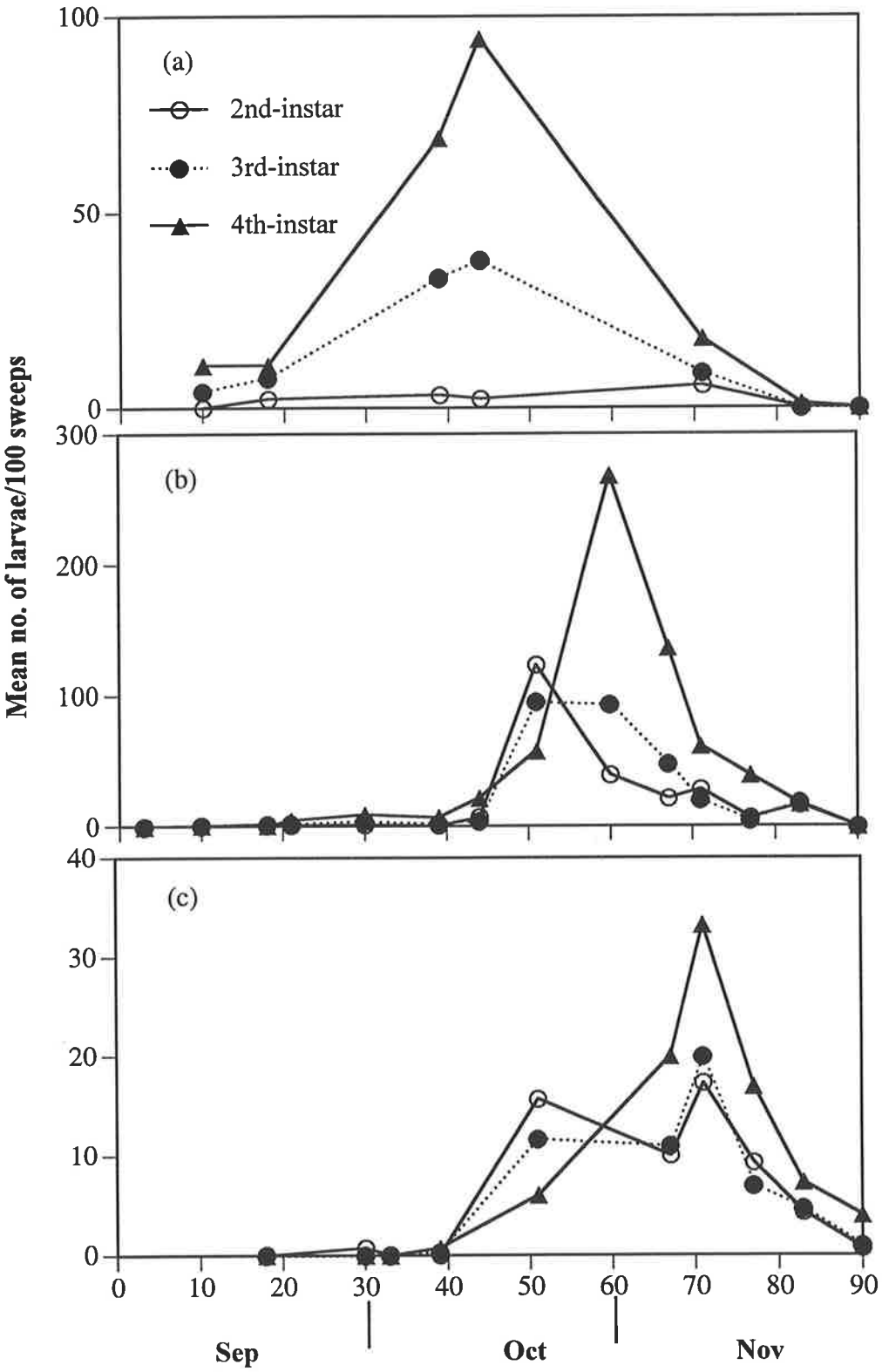


Figure 4.3. Age structure of *P. xylostella* (a) on wild mustard at Virginia; (b) on wild mustard in the northern Adelaide Hills; (c) on wild radish at Lenswood, 1994.

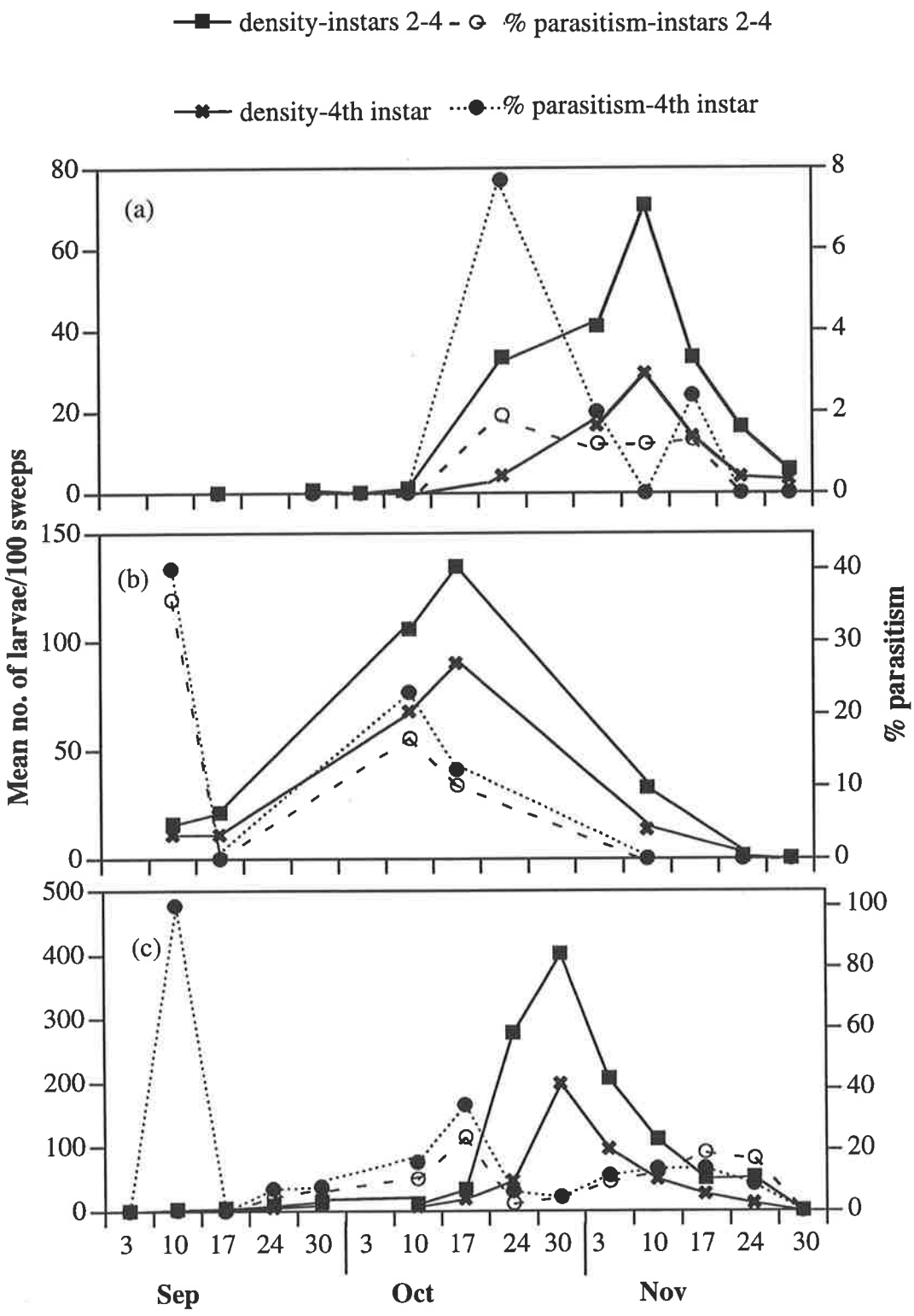


Figure 4.4. Larval densities of *P. xylostella* and% parasitism by *D. semiclausum* (a) on wild radish at Lenswood, (b) on wild mustard at Virginia, and (c) by *D. rapi* on wild mustard in the northen Adelaide Hills in 1994.

401 per 100 sweeps on the 28th of October and thereafter declined (Figure 4.4c). The last sample of DBM larvae on wild mustards was taken on the 25th of November when the plants had matured and they were poorly suited for DBM feeding. *D. semiclausum* was seen at all sites except the northern Adelaide Hills in 1994. In contrast, *D. rapi* and *A. ippeus* were abundant. Percentage parasitism by *D. rapi* peaked at nearly 24% on the 14th of October when larval density was low (Figure 4.4c). Of the 4th instars collected from mustards in the northern Adelaide Hills, the overall rate of parasitism by *D. rapi* was 9.2% (Appendix 7b).

Larval densities of DBM peaked at 134.3 larvae per 100 sweeps on mustard at Virginia on the 14th of October and declined thereafter (Figure 4.3a). The parasitoids *D. semiclausum*, *D. rapi* and *A. ippeus* were collected at this site. Percentage parasitism by *D. semiclausum* was approximately 10% on the 14th of October, the date of maximum larval density, but maximum parasitism was nearly 36% on the 10th of September when larval density was low (Figure 4.4b). Of 4th instar collected from mustards at Virginia, the overall rate of parasitism by *D. semiclausum* and *D. rapi* was 16% and 3.4% respectively (Appendix 7c).

The highest rate of parasitised larvae was found in fourth instars on all sample dates. In general the level of larval parasitism by the *Diadegma* species was not very high on weeds. Since the fourth instar represented the stage which accumulated parasitoid larvae of different instars it could be used as an indicator to estimate the impact of *Diadegma* species on DBM (see Chapter 3).

4.3.2. Unexplained larval mortality

In 1994, percentage mortality caused by unexplained factors varied markedly from month to month on weedy host plants (Table 4.1). This mortality in wild radish at Lenswood was 30.7% (range 0 to 55.1%), in wild mustards in the northern Adelaide Hills, was 25.2% (range 4.2 to 54.2%) and at Virginia, 10.0% (range 0 to 41.4 %) (Table 4.1; Appendix 7).

4.4 Discussion

The population densities of DBM larvae varied somewhat between surveys within each year and within each site. DBM larvae on the weeds were most abundant during spring and were relatively rare during the other seasons. In 1993 and 1994 DBM larvae were present

Table 4.1. Seasonal trend and percent unexplained mortality of larval DBM on weedy host plants in the Adelaide region, SA, 1994.

a) wild radish at Lenswood

Sample date	Total larvae /300 sweep	Total larvae died	% mortality
19-Sep-1994	-	-	-
27-Sept	2	1	50
5- Oct	-	-	-
10- Oct	3	0	0
24- Oct	100	47	47
4-Nov	123	39	31.7
10-Nov	212	45	21.2
14-Nov	100	20	20
22- Nov	49	27	55.1
28- Nov	17	7	41.2

b) wild mustards in the northern Adelaide Hills

Sample date	Total larvae /300 sweep	Total larvae died	% mortality
4-Sep-1994	-	-	-
10-Sept	6	1	16.7
16- Sept	11	2	18.2
23- Sept	24	1	4.2
30- Sept	43	2	4.7
7-Oct	31	2	6.5
14- Oct	99	11	11.1
21- Oct	830	134	16.1
28- Oct	1203	322	26.8
7- Nov	617	192	31.1
11- Nov	330	82	24.8
18- Nov	150	49	32.7
25- Nov	155	84	54.2

c) wild mustards at Virginia

Sample date	Total larvae /90 sweep	Total larvae died	% mortality
10-Sep-1994	14	0	0
18-Sept	19	0	0
9-Oct	95	4	4.2
14- Oct	121	12	9.9
11- Nov	29	12	41.4
23- Nov	1	0	0

on the weeds from early September. The relative larval density on weeds gradually increased from mid to late spring and peaked in late October to early November. This was followed by a rapid collapse of the larval population in late spring (November) (Figure 4.2a-c; 4.3a-c) which coincided with weed senescence and the onset of hot weather in summer. The distinct peak in larval densities in mid spring attributable to a relatively large number of 4th instars, occurred after the yearly peak of adults in mid spring (early to mid October) (Chapter 5). It is notable that the spring peak of relative larval density occurred first at Virginia in mid October 1994, later in the Northern Adelaide Hills, and last at Lenswood. This reflects the climatic differences among sites as Virginia experiences the highest temperatures and Lenswood the lowest.

Based on their incidence on wild radish and mustards, the most abundant alternative hosts, it was expected that DBM larval densities would differ between these wild host plants. DBM densities on wild radish were not as high at Lenswood as they were in the northern Adelaide Hills at the same time. The areas for larval sampling on wild radish and mustards were almost of the same size in 1994. However, the results of this study showed that the total relative larval densities on mustards in the northern Adelaide Hills were approximately 6 times higher than that on wild radish at Lenswood. Since plant diversification influences the population dynamics of insect herbivores in agricultural (Root 1973; Risch *et al.* 1983; Andow 1988) and natural habitats (e.g., Kareiva 1983; Stanton 1983), many smaller plantings of brassicaceous crops in the northern Adelaide Hills may have contributed to the greater population size observed at this site. However, climatic conditions (hot summers) influence plantings of crops in this region. It is suspected that the abundance of host plants was a significant factor in the larval population differences observed in the two locations. The northern Adelaide Hills, located 15 km to the NE in urban areas, contains many small brassicaceous croping fields. These fields were spread among private owners. Most of the fields were well drained. Therefore, brassicaceous plants and growing sites (e.g., fence rows) for wild mustard plants were more available to sustain DBM populations in spring in the northern Adelaide Hills than at Lenswood.

This study suggests that brassicaceous weeds serve as potential alternative host plants for the DBM activities in winter and early spring when the suitable host crop plants are not available. This is in accordance with Harcourt (1957) who indicated the importance of

wild host plants in the absence of cultivated crucifers in early spring. However, in the Adelaide region, all alternative host plants dry off by late spring and are not available for DBM in summer. This lack of suitable host plants together with the adverse physiological effects of high temperature in summer (Wakisaka *et al.* 1992) are the likely causes of low DBM activity during this period. However this is similar to the cause for the marked summer decline of DBM that happens in warm temperate areas of the central and southern parts of Japan (Koshihara 1986). After early autumn in Adelaide when fresh wild host plants gradually become available again, DBM larval densities increase but only to low levels. Accordingly, there is relatively little parasitoid activity observed during this period. Since DBM does not diapause in the Adelaide region (Atwal 1955), its development continues slowly even in winter. It is suspected that winter rainfall limits populations of DBM. In spring, DBM developmental time decreases, wild host plants become widely available and cultivated areas of brassicaceous crops gradually increase. Local populations of DBM on weeds are likely to serve as a source of reinfestation on newly planted crops in spring. In field boundaries, roadside verges and other unploughed areas containing wild radish and/or wild mustards, DBM may be present in relatively high densities. From such small areas the DBM and its parasitoids may spread to adjacent fields. The areas covered by wild brassicaceous plants in the Adelaide region are probably also invaded by migrating DBM adults in early spring. Larvae collected feeding on weeds in late October and early November in the Adelaide region are probably from local populations combined with the offspring of migrating DBM adults from more distant over-wintering sites (Chapter 5). Parasitoids activity is low in the early spring on wild host plants. In general, increasing areas of weeds and brassicaceous crops, may imply increasing population densities of the DBM and thus risk of heavy damages in attractive crops.

Overall, mortality was mainly attributable to unexplained mortality factors which occurred predominantly during the young stages (Appendix 7). Those individuals which showed possible symptoms of viral or fungal disease were suspected of being diseased larvae. The level of mortality varied from month to month and was greatest in late spring. Regular rainfall may promote diseases in the spring.

In 1994 there was an apparent peak in the rate of parasitism in September at Virginia and the northern Adelaide Hills. This peak could have been due to sampling error when initial densities were low (Figure 4.4b-c). Regardless of whether the samples were taken from

wild mustards or wild radish, both DBM and parasitoid densities increased throughout the spring, particularly from mid October onwards at all locations. In general, *Diadegma* species were rare in September and October. In this study the level of parasitism of the DBM larvae on wild radish and wild mustards in spring was low for the three parasitoid species *D. semiclausum*, *D. rapi*, and *A. ippeus*.

In general, the results suggested that the important limiting factors for survival of DBM are unexplained mortality factors, parasitoids during spring (all three species *D. rapi*, *D. semiclausum*, and *A. ippeus* on weeds but mainly the two latter on crops (Chapter 3)), and a combination of warm and dry weather, weed senescence in late spring, and crop maturity in summer (Chapter 3). In addition, alternative weedy host plants are limiting in autumn, and the cold and wet weather in winter slows development and increases larval mortality.

Parasitoids accounted for very little of the mortality of DBM larvae on weeds. DBM larval parasitoids especially *D. semiclausum* were not numerous on weeds in either year. In 1993 and 1994 parasitism contributed little to mortality early in the spring season. Although *D. semiclausum* and *D. rapi* were found at Lenswood in 1994, the percentage parasitism peak of *D. semiclausum* was extremely low (1.9%) whereas the percentage parasitism peak of *D. rapi* in the northern Hills was 23.9% (Figure 4.4a;c). *D. rapi* was also found at the other study sites during 1994 but only at relatively low abundance. It is not clear why *D. rapi* was the only ichneumonid parasitoid found in DBM larvae on wild mustard plants in the northern Adelaide Hills. It is possible that because this species is an indigenous parasitoid of DBM larvae in Australia (Gauld 1984), its distribution extends into many areas including the hills areas. This area was not sampled in 1993. A potential explanation for the apparent lack of *D. semiclausum* in the northern Adeliade Hills in 1994 might be that a host is sometimes attacked by a parasitoid species in one region and by a different parasitoid or perhaps not at all in another region (Vinson 1984b). Additionally, it could be that *D. semiclausum* was in fact present in the northern Adelaide Hills but this occurred at such low densities that the sampling method used could not detect its presence. Furthermore it appears that parasitoids like *D. rapi* recolonise some areas periodically. In some years they colonise an area but in other years they do not, and therefore the importance of parasitoids varies from year to year.

The level of parasitism by *D. semiclausum* was likely to be too low to control the DBM, in the horticultural area of Virginia where cabbage producers frequently applied insecticides (two or three applications per week). Since *D. semiclausum* was observed and collected

from the weeds in the period of insecticide application in these areas, it is possible that *D. semiclausum* has developed some resistance towards the insecticides and has adapted well to this kind of environment. Sastrodihardjo (1986) also found a degree of resistance to insecticides of *D. semiclausum* in Indonesia. Although the levels of parasitism by *D. semiclausum*, *D. rapi* and *A. ippeus* in the present study were not high, each of these parasitoids, can be considered as a factor contributing to the mortality of DBM on weeds. It may therefore be usefull to try to conserve these natural enemies by either removing or decreasing adverse factors that might have a deterimental impact on the parasitoids (Debach and Rosen 1991).

Percentage parasitism from density samples gave mean percentage parasitism of *Diadegma* species for all sample dates, which may underestimate parasitoid impact in some situations (van Driesche 1983). *Diadegma* species emergence from DBM larval instars found in the field revealed that oviposition of the parasitoids must have taken place in different larval stages. Laboratory experiments (Chapter 6) showed that *D. semiclausum* oviposited directly into different larval instars but preferred younger instars, mainly the second and third instars as the most preferred hosts. Under field conditions, the parasitised and unparasitised fourth instar larvae remain longer than the other instars and were therefore relatively more abundant in samples. This factor lead to an overestimation of the percentage parasitism for fourth larval instar (Waage and Cherry 1992). However, because fourth instar represented the stage which accumulated parasitoid larvae of different instars, the number of parasitised fourth instars was taken as an index of DBM parasitism (see Chapter 3).

In summary, this study, which was conducted at four field sites in South Australia: two on the Adelaide plain (Turretfield Research Centre and Virginia) and two in the Adelaide hills (Lenswood and the northern Adelaide Hills) provided regional information about the distribution and abundance of DBM and its parasitoids. Data of this nature gives valuable information about the local importance of DBM and its related host plants in a specific vegetable-growing area. The timing and magnitude of population peaks (Figure 4.4), and annual recolonisation of parasitoids and/or DBM show that there is temporal variation in size of populations of DBM and its parasitoids from site to site. Therefore the focus of IPM for DBM should be local rather than regional.

CHAPTER 5

ADULT ACTIVITY OF DIAMONDBACK MOTH IN THE ADELAIDE REGION

5.1. Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.), is a highly mobile and serious insect pest of brassicaceous vegetables and is present in almost all parts of South Australia. Because of insecticide resistance, DBM control is difficult in Australia (Altmann 1988) as well as in many countries of Southeast Asia (e.g., Cheng 1986; Sun *et al.* 1986; Miyata *et al.* 1986). DBM resistance to insecticides has also been reported from South Australia (Baker 1994). In order to establish an effective control program, it is important that the dispersal distance of DBM and factors influencing its flight behaviour on crop fields are understood (Tabashnik *et al.* 1987; Shirai and Nakamura 1994). In the Adelaide region of South Australia, a large number of adult DBM and immature stages commonly have been observed in the spring but only small numbers during other seasons. It appears that DBM populations in spring may have been produced by local adults that were produced on brassicaceous weeds in the Adelaide region. However, to date there has been no critical examination of the local dispersal and/or migration pattern of DBM in South Australia.

In this study, traps containing synthetic female DBM sex pheromone were used to monitor the seasonal activity of DBM. A number of studies have proved the activity of the pheromone as well as its potential application to DBM monitoring programs (e.g., Chisholm *et al.* 1979; Baker *et al.* 1982). The sex pheromone trap is an appropriate tool for sampling (Daly and Fitt 1993), detecting and estimating population peaks (Chisholm *et al.* 1979), and in some cases an effective measure for forecasting occurrence (Shirai and Nakamura 1995).

The Adeliade region is characterised by a 5-month dry season (November-March) and most of the rain falls from late April through September each year. Although some rain usually falls from November to March, it is generally light and variable (South Australian Year Book 1995). Mean annual rainfall ranges between 500 and 1200 mm. The seasonal changes of numbers of male DBM caught by the pheromone traps adjacent to the rapeseed,

Brassica napus L. cv. 'Rangi', and kale, *Brassica oleracea* L. var. *acephala* cv. 'Grüner' fields described in Chapter 3 were investigated. DBM oviposits adventitiously on any available brassicaceous host plants. Estimating population densities and also short-distance movement of DBM in spring from brassicaceous weeds to new crops, based on males trapped is impossible. Therefore, records of eggs laid on sentry plants grown in a glasshouse and placed in the field were collected over a 19-week period in spring-summer of 1994-95 at Lenswood. The possibility of spring dispersal of DBM in the Adelaide region was also investigated.

5.2. Materials and methods

5.2.1. Pheromone trap survey

The presence and abundance of DBM males through their response to a synthetic female sex pheromone was monitored. Seasonal changes of the number of DBM adults caught by the pheromone traps were investigated in a total of three different study sites in the Adelaide region. DBM was sampled in traps adjacent to brassicaceous crops (see Chapter 3 for details) between 21 August 1993 to 24 December 1995 at Lenswood , near weeds between 22 August to 11 December 1993 at Turretfield (see Chapter 4 for details), and at a residential area at Flagstaff Hill (S 35 ° 03´, E 138 ° 35 ´) a suburb of Adelaide (Figure 3.1), from 26 March 1994 to 29 December 1995. Lenswood was characterised by large patches of wild radish *Raphanus raphanistrum* L. and sparse vegetation of brassicaceous crops especially cabbages. Turretfield is primarily a pasture area where the dominant weed species were wild mustards *Sisymbrium* spp. At Flagstaff Hill the trap was placed in a private yard. Nearby properties had orchards and ornamental plants.

At each plot or study site one synthetic sex pheromone trap was set. However, at least three traps were simultaneously used with 500-700 m spacing between trap sites from March 1994 onward at the Lenswood location. A rubber septum impregnated with synthetic sex pheromone was suspended 10 cm above the surface of sticky inserts in AgriSense Delta Traps (AgriSense-BCS Limited, UK). No information about the ratio of components could be obtained from the manufacturer. In the field each trap was set at a height of about 100 cm, approximately 50 m from the edge of the field plots. Pheromone dispensers were exchanged every 4 weeks when large numbers of DBM were captured, or

if the sticky inserts deteriorated and every 6 weeks in remainder of the year. These traps were routinely checked once a week, unless otherwise stated.

Occurrences of adult population peaks for each year were determined from pheromone catches. Sampling dates were considered to be the mid-point of the weekly interval, and data are shown by three-point moving averages of log DBM/trap/day. This was done to give a visualisation of the temporal pattern of trap catch. As the sampling dates were arbitrary, a shift of several days could change the pattern of trap catch if a moving average was not used.

5.2.2. Sentry plant survey

A modified "rope wick technique" (Marrone 1982) was used for maintaining moisture in pots for a 7 day period while containing plants (Figure 5.1). This technique was applied for use in studies on oviposition by DBM females, which required growing kale plants in the pots and in the field conditions. Plastic pots of two different sizes were used. The smaller pots were 15 and 20 cm in diameter at base and top respectively, and 14 cm deep. The larger pots were 15 and 20 cm in diameter at base and top respectively, and 20 cm deep. Each small pot was filled with UC soil and kale seeds were sown. Pots had two holes, 1.5 cm in diameter, in the bottom at different sides. A cotton rope, ca. 1 cm in diameter and 60 cm in length, was inserted into each pot through one hole in the bottom, passed through the soil close to the top of the pot over a wooden block placed in the middle of pot (8 cm × 6 cm × 4 cm), and came out through the other hole. About 10 cm of rope was hanging down on each side. A plastic bag was placed inside the larger pot and was filled with water such that the rope of the planted pot extended 10 cm into the water. By this technique, moisture was provided by the rope close to the plant's roots. Seven pots of one and half month old plants of kale (glasshouse reared) were placed in scattered locations between a relatively large area of wild radish and kale plots at Lenswood Research Centre once a week for 19 consecutive weeks. The number of eggs and larvae on sentry plants were counted following their removal from the field and were used as an indicator of the eggs oviposited by DBM females.

Figure 5.1. The rope wick system for maintaining moisture in pots in the field.

Data in each collection period from pheromone trap and sentry plants were transformed (log 10 [1+x]) to stabilise variance (Zar 1984), and three-point moving averages were used to visualise seasonal trends.

5.3. Results

Pheromone traps

DBM appeared to be highly seasonal, and the temporal distribution varied from site to site. Two features were consistent in this pattern: (1) very few moths were collected from January to early March, the months of the dry season, or from June through August, the wettest and coldest months, and (2) the numbers of DBM adults trapped was greatest from late August to November.

In each year the majority of adult male DBM were caught in pheromone traps from August to late December at Lenswood. Traps showed that moths were in flight all year round, however, few moths were caught between January and August (Figure 5.2).

Weekly records for DBM collected in the traps indicated that the moth was not present in equal abundance throughout the year (Figures 5.2-5.4). Usually but not always the number of moths remained at a lower level in September compared to October and November.

The first trap catches at Turretfield started in August 30 and finished by November 20 in 1993. Weekly records for DBM males collected in traps at Turretfield indicated that the DBM flight pattern was similar to that at Lenswood, although the numbers trapped were less at this location (Figure 5.3). At Turretfield from late November 1993 until April of the next year no moths were trapped.

The first DBM males were recorded at Flagstaff Hill on September 1 and peaked in late September. Although from late August to late December the total number of adults at Flagstaff Hill were fewer than Lenswood, the pattern of abundance was similar (Figure 5.4). From 29 December 1994 until April of the next year no moths were trapped at this location.

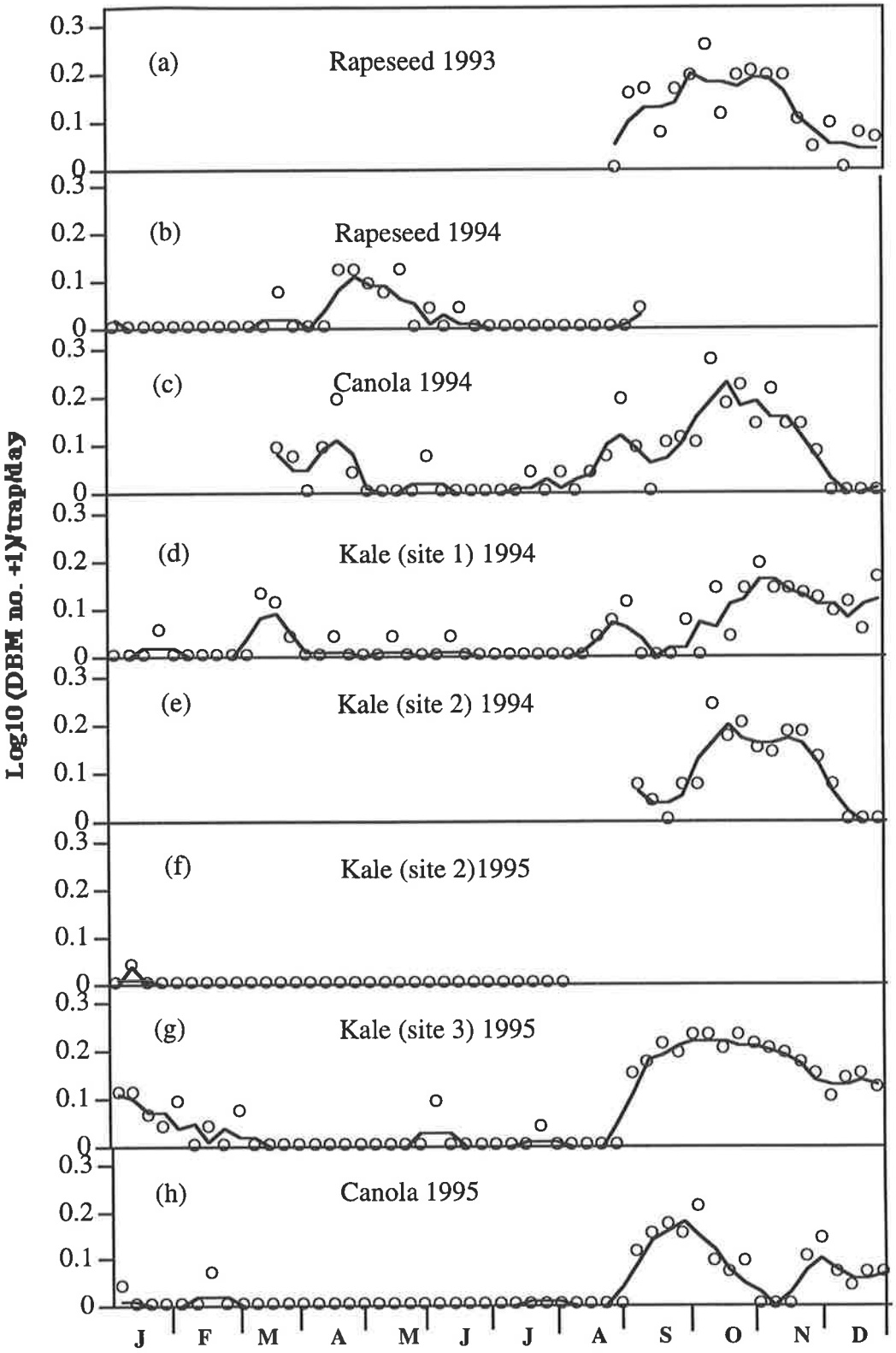


Figure 5.2. Seasonal distribution of male DBM attracted to sex pheromone traps at Lenswood, SA. Plotted points show data (log10 (DBM+1)/trap/day) and solid lines are three-point moving averages.

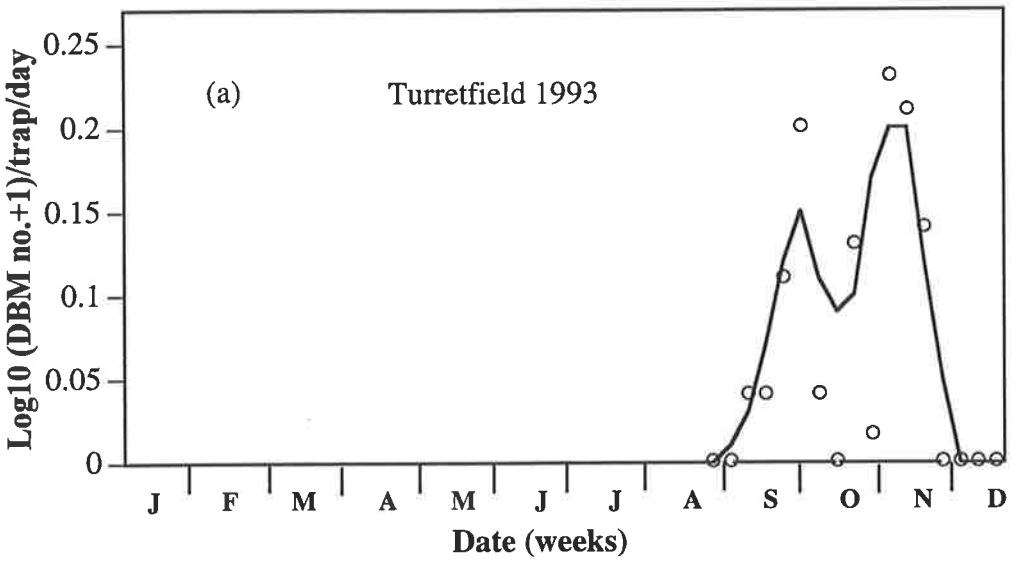


Figure 5.3. Seasonal distribution of male DBM attracted to sex pheromone traps at Turretfield, SA. Plotted points show data (log10 (DBM+1)/trap/day) and solid line is three point moving averages .

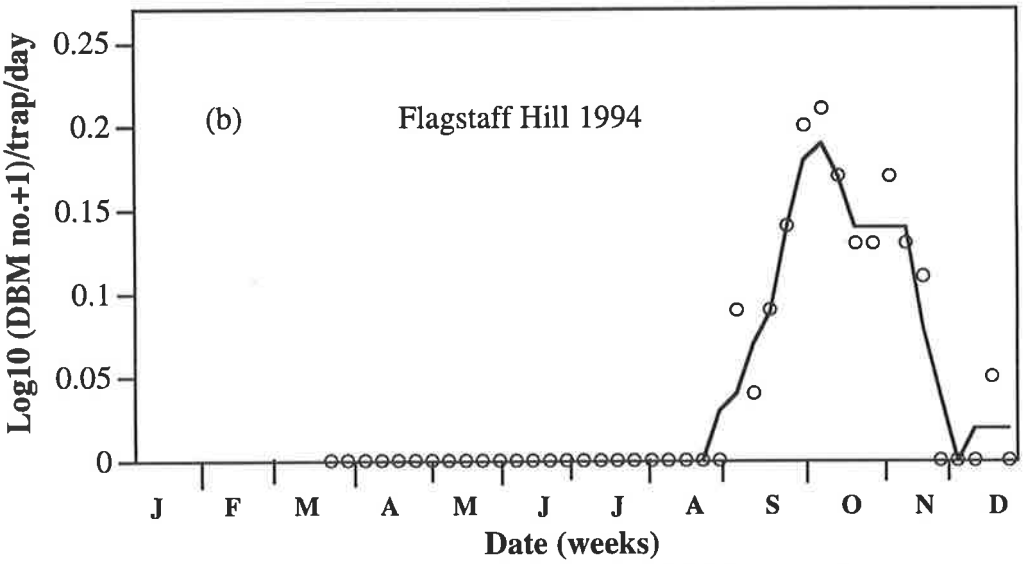


Figure 5.4. Seasonal distribution of male DBM attracted to sex pheromone traps at Flagstaff Hill, SA. Plotted points show data (log10 (DBM+1)/trap/day) and solid line is three point moving averages .

Sentry plants

The abundance of trapped moths in October did not follow oviposition by DBM females, as a paucity of eggs was observed on kale sentry plants until November (compare Figure 5.2a-e with Figure 5.5). However, one peak of oviposited eggs was observed on sentry plants in late December.

5.4. Discussion

The seasonal pattern of DBM in the Adelaide region (Chapters 3-4) derives from local population activities in spring and during the remainder of the year. Its seasonality is probably due to local dispersal and possible DBM migration. Late spring-early summer movement probably occurs, but cannot be detected because there are local sources of moths. The fact that DBM is present during the "off peak" months, albeit in low numbers, indicates year round activity of DBM in the Adelaide region. The relatively low numbers of DBM adults detected in early spring and those that emerged from October to late November suggest that they are from local DBM populations. These populations were detected by pheromone traps (a very highly sensitive technique) throughout the year (Figures 5.2-5.4). Trap catches showed one broad peak from August to December in 1993 (Figure 5.2a), two peaks, one in late August and the other from late September to November in 1994 (Figure 5.2c-e), and one peak from September to December in 1995 (Figure5.2g-h). In 1994 great variations were attributable to variable climatic factors. In 1995 canola plants were cut off in November and this probably caused a sharp decrease of male DBM catches in pheromone traps adjacent to the field (Figure 5.2h). In summer high temperatures, which prevent egg laying by DBM, shortage of available suitable host plants, and the high activity of parasitoids in late season (Chapter 3) are factors that probably have impact on the DBM populations at this time of the year, and thereby limit its ability to substantially increase in abundance.

It was not possible to explain the relatively high numbers of DBM adults trapped in October. Low larval density of DBM in early to mid spring (September to late October) was found by intensive larval sampling once per week throughout the year (Chapters 3-4). Also very low numbers of eggs were laid on sentry plants in early spring and low numbers

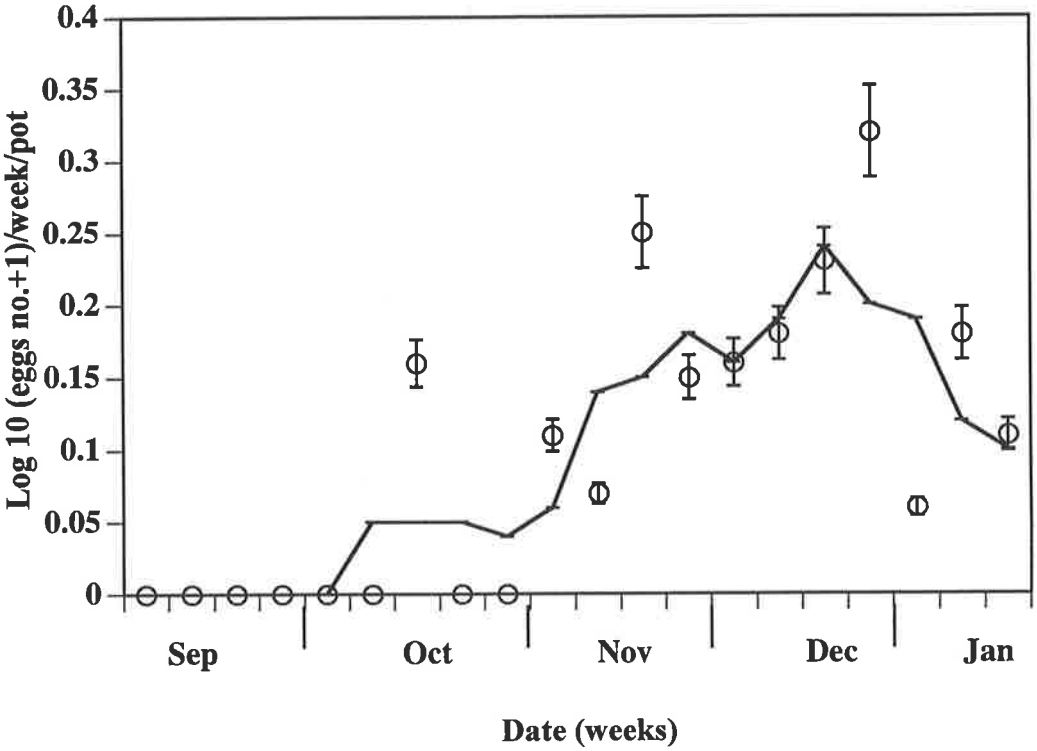


Figure 5.5. Number of eggs oviposited by DBM adults on kale sentry plants at Lenswood, SA, 1994-1995. Vertical bars indicates standard error of means. The solid line is a three-point moving average.

of adults were caught in pheromone traps in winter and early spring (Figures 5.2-5.4). These trends suggest that adult DBM may have moved into the Adelaide region from outlying winter habitats.

Population densities (Wedding *et al.* 1995), the effects of seasonal variations of environmental conditions (Cardé *et al.* 1975; Maa 1986; Grant *et al.* 1996), and endogenous factors including the genetic differences between DBM in individual field populations (Maa 1986) influence adult moth response to the pheromone. Fluctuations in trap catches in the Adelaide region may be influenced by stochastic effects of weather and climate, larval and/or pupal mortality, and also DBM movement. However, it seems that pheromone trapping is a very efficient and sensitive indicator for the presence of DBM (as indicated by an increase in the number of trapped males in October) in comparison with the other methods, e.g., conventional density sampling methods (chapter 3), and sweeping (Chapter 4).

DBM adults were most abundant in spring. However, they laid no eggs on sentry plants during September and only low numbers of eggs in mid October (Figure 5.5). Rainfall causes high DBM mortalities (Harcourt 1963). One possibility is that stochastic events such as local heavy rain, wind and storm suppress DBM populations. Another possibility is that low numbers of eggs may be attributable to very low numbers of possibly migrant ovipositing adults. It is also possible that migrant DBM may have lower fecundity, an observation consistent with many other insect species (Roff and Fairbairn 1991). The source of these DBM adults are most likely wild brassicaceous plants, wide spread in all geographical areas of South Australia. Although the present study demonstrated an almost simultaneous wave of adult flight in the fields in different areas of the Adelaide region in spring, the evidence for DBM movement is not conclusive.

Data from larval sampling, adult trapping with pheromones and oviposition on sentry plants combine to indicate the phenology of DBM in the Adelaide region. DBM exploits its wild hosts for a relatively long period each year, from early autumn to late spring according to climatic conditions. In early spring, before the brassicaceous crops are available, wild brassicaceous plants, particularly flowering wild radish and wild mustards play an important role in maintaining a small DBM population. This is consistent with

information reported by Marsh (1917). During mid-spring whenever cultivated plants are available, DBM simultaneously exploits weeds and its cultivated hosts. However, the appearance of DBM adults (Figures 5.2-5.4) in the Adelaide region revealed that DBM often occurred in high numbers on brassicaceous plants in spring. These DBM combined with populations in field boundaries, roadside verges and other unploughed areas.

In late spring there is a marked deterioration of weed availability and quality in the Adelaide region. At this time local dispersal is likely to occur to more favourable fields or areas where cultivated crops are available

After weeds developed during spring, large numbers of DBM moved into crops and caused serious damage. As the weeds began to senescence and became undesirable to DBM larvae and adults in late November and early December, they almost disappeared and their population decreased. However, during the summer a very low population of DBM occurs on crops and movement from one crop to another may occur.

Adults that emerged in summer oviposited on brassicaceous crops that were still available. For example, kale plants in the field were cut off in mid December 1994, and regrowth was examined for eggs in early January 1995 when the height of plants was between 10 to 30 cm. All stages of DBM were observed at this time. Therefore, DBM populations are able to persist during summer on crops. Thus, DBM adults which are present throughout the year appear to oviposit adventitiously on any available brassicaceous plants.

The DBM population during autumn and winter was extremely low. New weeds which germinate with the first rainfall in autumn provide important refuges for the DBM which disperses from crops. The DBM persists in low numbers on weeds in winter.

In summary, local DBM populations in the Adelaide region are enhanced due to more favorable conditions in early spring, i.e., an abundance of fresh wild host plants and brassicacoeus crops, increasing temperatures and decreasing rainfall.

CHAPTER 6

PARASITISM BY *DIADEGMA SEMICLAUSUM:* EFFECTS OF DIAMONDBACK MOTH LARVAL INSTARS

6.1. Introduction

Insect hosts with overlapping generations provide a range of host stages for their parasitoids to attack at any one time. The range of host stages encountered will influence host utilisation by parasitoids (Charnov and Skinner 1985). The chance of encountering different host stages may lead to differential attack (e.g., Price 1972; van Alphen 1980; Liu *et al.* 1984).

Different levels of parasitism may be due to variation in the acceptance of host stages by parasitoids. Many insect parasitoids have been shown to prefer particular host instars (Vinson 1976). Host instar preference may be influenced by changes in the physiological state of the parasitoid (Weisser 1994). Other factors such as host size (e.g., Opp and Luck 1986; Reeve 1987; de Jong and van Alphen 1989), age (Juliano 1982), morph (Liu *et al.* 1984; Lardner and Hales 1990), and stage of development (Hopper 1986) influence host acceptability. The oviposition ability of parasitoids may be reduced by physical defences of the host (Taylor 1988; Allen 1990). In addition, the parasitoid's egg supply (Bartlett 1964; Rosenheim and Rosen 1991), age (van den Assem *et al.* 1984; Avilla and Albajes 1984; Wong *et al.* 1990), level of previous host experience (e.g., van Lenteren 1976; Charnov and Skinner 1985) and mating status will influence its oviposition activity. *Diadegma semiclausum* is reported to parasitise all larval instars (Chua and Ooi 1986), but it preferentially attacks the 2nd and 3rd instars of DBM (Lloyd 1940; Velasco 1982). However, Talekar and Yang (1991) reported that 4th instars are never attacked. Experiments were undertaken to determine the host stage preference of this parasitoid and to resolve this discrepancy.

6.2. Materials and methods

6.2.1. Rearing procedure for host larvae

The larvae used in the experiments came from a laboratory colony of diamondback moth that have been reared for about 6 years. The food plant was cabbage, *Brassica oleracea* L. cv. 'Green Cornet' which was available throughout the year. On reaching the adult stage, moths were transferred to an oviposition cage where they were allowed to mate and to oviposit on leaves of cabbage. The adults were provided with drops of pure honey and cotton wicks saturated with water. Plants with the eggs on their leaves were kept at 25°C. Whenever a large supply of different host instars were needed, rape plants (*Brassica napus* L. cv. 'Rangi') were grown in vermiculite using the method of Liu and Sun (1984). Eggs deposited were allowed to hatch at room temperature and fed on host plants. Soon after all instars were available, the larvae were transferred onto potted rapeseed or cabbage plants in numbers required for use in experiments. Larval instar was identified by the width of head capsule (Herminanto 1995).

In order to obtain first instar of DBM, a cylindrical glass tube (2 cm diam. × 13.5 cm length) was used as an oviposition chamber (Atwal 1955). The ends of the tube were closed with corks. An oviposition substrate was provided by a disk of cabbage leaf over a piece of tissue paper that was held over each cork with muslin cloth. Five to 10 pairs of adults were placed into each tube to lay eggs. Each tube was supplied with a piece of wet cotton wool or cotton wick to which a few drops of honey were applied. DBM females laid eggs quite readily on muslin inside or even on the cylindrical walls of tubes. After egg hatching in about 4 days, the first instar larvae were transferred onto potted cabbage plants for use in experiments.

6.2.2. Parasitoids rearing procedure

A stock culture of *D. semiclausum* Hellen (Hymenoptera: Ichneumonidae) was established from parasitised larvae and pupae originally collected from a kale, *Brassica oleracea* var. *acephala* L. cv. 'Grüner', field at Lenswood in the middle of December, 1993. Adult parasitoids were housed in a cage and allowed to mate freely. Each cage was 30 cm × 30 cm ×30 cm with three sides of each cage covered with a gauze fine enough to prevent the escape of host larvae as well as parasitoid adults. The top was covered with Perspex

permitting visual inspection and the bottom was sheet metal. A closely fitting sleeve with cloth screen on one side formed the door. The wasp adults in the cage were provided with drops of honey and a cotton wick saturated with water. Wasps were maintained on larval DBM on potted cabbage plants. The cages were kept at 24±1°C under a photoperiod of (14L:10D) hr.

6.2.3. Experiment 1

Experimental cages were 60 cm × 60 cm ×60 cm. Three sides of each cage were covered with a muslin-screen, the top was covered with Perspex pane permitting visual inspection and the bottom was plywood. A sliding Perspex pane on one side formed the door. Six potted cabbage plants were placed in each cage. Three of the plants were colonised with 24 second, third, or fourth instar larvae only, with one such plant per cage. Three more potted cabbage plants carried 8 larvae of all three instars for a total of 24 per pot. The DBM larvae were exposed to parasitoid oviposition for 24 hr. In many studies, responses to odours from the food of the host have been shown to be essential in host location (e.g., Dicke *et al.* 1984; Vet and van Opzeeland 1985). Therefore, one night before the experiment, the larvae were placed on the each host plant to commence feeding and produce chemical cues for parasitoid activity.

Four days after their eclosion (= 4 days old), five adult female *D. semiclausum* were released at 11 am, and parasitoids and larvae were removed at 11 am following day. After this exposure, each larva was collected separately from the plants and transferred into a plastic cup (7 cm diam. × 5 cm h.) provided with a ventilated lid. All larvae were fed with fresh leaves of cabbage and maintained at 24±1°C until parasitoid and/or DBM adults emerged. Since some larvae were lost in handling, parasitism rates were calculated from the net numbers of DBM larvae that pupated. At the pupal stage, the number of parasitised larvae was determined by the presence of parasitoid pupae. *D. semiclausum* cocoons appeared dark with a pale transverse band and were seen inside DBM cocoons as the pupal period progressed. This experiment with 2 cages was replicated 2 times.

6.2.4. Experiment 2

Levels of parasitism on all larval instars of DBM were determined in the second experiment. Larvae were reared on cabbage plants and presented as a single instar on each

plant, with all four instars represented in each cage. On the night before the experiment, larvae were placed on each host plant to produce chemical cues for parasitoid activity.

Four potted cabbage plants, 13-15 cm h and with 5 leaves, each with 20 first, second, third, or fourth instars were placed in each cage.

Four 6-day old female *D. semiclausum* that were fed with honey and water were introduced into each of four cages (60 cm × 60 cm × 60 cm). Wasps were released at 9 am, with parasitoids and larvae removed at 9 am following day. After this exposure, the plants were moved out of the cage, first and second instar larvae were maintained on the host plants at 24±1°C until they developed to 3rd and/or 4th instar larvae. Third and fourth larval instars were collected separately from the plants and were then transferred into a plastic cup (7 cm diam. × 5 cm h.). These larvae were kept at −20°C until dissected to determine the numbers of parasitised larvae. Since some larvae were lost in handling, parasitism rates were calculated from the net numbers of DBM larvae collected. The larvae were dissected under methylene blue and isotonic saline solution, or tap water using a stereomicroscope. With experience, parasite larvae are readily detected at magnifications of 12 to 40×.

6.2.5. Analysis of data

All data were expressed as percentage parasitism and analysed by analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (SAS institute 1993). The means of three last larval instars were compared using Tukey's Studentized Range (HSD) test for variable and that of all larval instars by Studentized Newman-Keuls test for variable at $P \leq 0.05$. In the 1st to 4th larval choice experiment, percentage larval parasitism was arcsine transformed prior to analysis of variance to stabilise variances.

6.3. Results

6.3.1. Experiment 1

D. semiclausum oviposited on 2nd, 3rd, and 4th-instars, but the second and third instar were preferred. Levels of parasitism were higher for 2nd and 3rd instars without regard to whether only one instar or a mixture of all three were present on individual plants (Figure 6.1; Table 61.1a).

6.3.2. Experiment 2

When exposed to all instars, parasitism remained relatively high for larvae of second and third instars, but was lower on first and fourth larval instars (Figure 6.2; Table 6.1b). Parasitism was significantly different among all instars of DBM (P= 0.0001). Mean comparisons revealed that most parasitism occurred on second instars, less on third instars and least on 1st and 4th instar larvae respectively. Comparisons of percentages of larvae that were lost showed that there were no significant differences on establishment of larval instars.

6.4. Discussion

The results indicated that there was a significant difference in levels of parasitism on all host stages by *D. semiclausum* in choice experiments. However, all four host stages were attacked and greater parasitism of 2nd instar hosts than 1st, 3rd, and 4th instars was observed. This indicates that parasitoids responded differently to each host stage. These results do not agree with the findings of Talekar and Yang (1991) who reported that *D. semiclausum* failed to parasitise the 4th instar larvae of DBM.

Host selection by a parasitoid is affected by both physical and chemical factors (Vinson 1976; Sands 1993). Physical host defences may also reduce the success of parasitoid oviposition (Taylor 1988; Allen 1990). Head-thrashing, rapid wiggling, extrusion of fluid droplets directed at the attacking female parasitoid, and encapsulation and other immune responses against non-self substances are active defensive responses against parasitoids at the behavioural and physiological levels (e.g., Vinson and Iwantsch 1980; Slansky 1986). Some hosts are attacked because they are accessible in a particular habitat by a female, not because they are preferred (Vinson 1976). Wriggling behaviour and/or dropping from the leaf on a fine silken thread by 4th instars could reduce the efficacy of parasitoid attack, or at least, prolong the time needed to successfully oviposit, thereby limiting the level of parasitism. By contrast, physical defence by DBM first instar larvae is limited by its leaf mining habit.

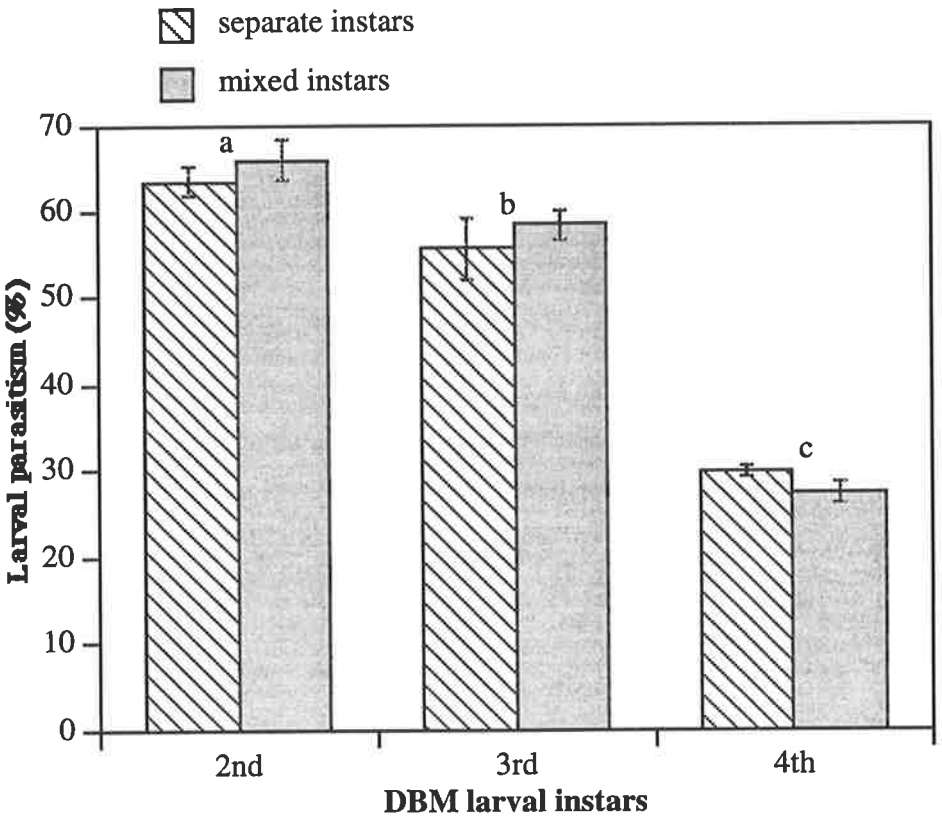


Figure 6.1. Parasitism of 2nd-, 3rd-, and 4th-instars of DBM larvae by *D. semiclausum.*, with separate instars or mixed instars on cabbage plants. Error bars are mean ± SE. Different letters above columns indicate statistically significant differences ($P<0.05$, Tukey HSD).

Table 6.1. Analysis of variance for parasitism of instars of DBM larvae by *D. semiclausum.*

a) 2nd-, 3rd-, and 4th-instars, with separate instars or mixed instars on cabbage plants.

Source of variation	Degrees of freedom	Mean squares
Instar	2	1450.96***
Cage	1	2.25
Instar x Cage	2	8.38
Residual	6	8.40

*** Significant at $p < 0.001$.

b) 1st-, 2nd-, 3rd-, and 4th-instars, in caged choice tests.

Source of variation	Degrees of freedom	Mean squares
Instar	3	288.35***
Residual	12	4.80

*** Significant at $p < 0.001$.
Based on acrsine transformed data.

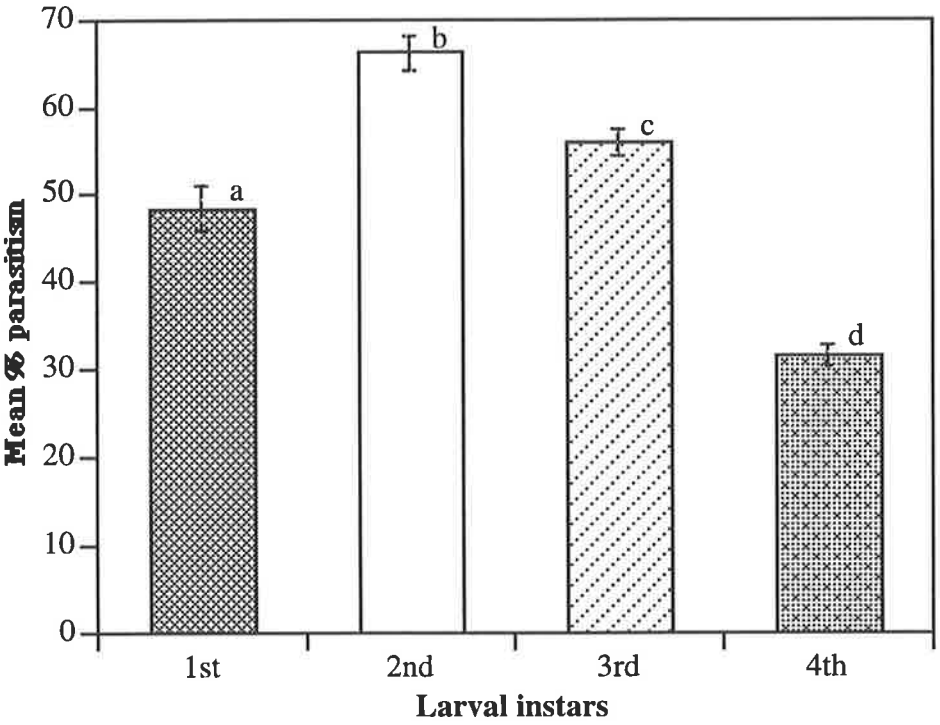


Figure 6.2. Parasitism of 1st-, 2nd-, 3rd-, and 4th-instars of DBM larvae by *D. semiclausum* in caged choice tests. Error bars are mean ± SE. Different letters above columns indicate statistically significant differences ($P<0.05$, Studentized Newman-Keuls).

Another important factor is the amount of nutrients available to a parasitoid (Vinson 1984a). Food and living space for larval parasitoids (endoparasitic insects) and sometimes food for the adult parasitoid are provided by insect hosts (Slansky 1986). For many species of parasitoids, such as parasitic Hymenoptera, host quality is assumed to be determined by host size (Opp and Luck 1986; Vinson and Iwantsch 1980; Kouamè and Mackauer 1991). In other words, the size of the host insect attacked often determines the quality of a host or the amount of food available to developing parasitoids (Vet *et al.* 1993). However, host quality is one of many factors that determines oviposition decision by insects (van Alphen and Vet 1986). Askew and Shaw (1986) noted that host quality is evaluated generally with size in hosts, such as eggs and pupae, that do not feed or grow during parasitism. In contrast, Sequeira and Mackauer (1994) pointed out that at parasitisation, quality for the immature parasitoid in larval hosts that continue to feed and grow during parasitism may vary with the host stage, rather than with size. Small hosts may be less favourable for parasitoids because they produce less fit offspring (or more males) (Vinson and Iwantsch 1980; Vinson 1984a). Talekar and Yang (1991) pointed out that *D. semiclausum* ovipositor is as large or larger than the diameter of the first instar larvae, and this may restrict egg laying in the first instar.

The larvae of first instar DBM, although usually within the tissues of the food plant, were parasitised by *Diadegma insularis* (Cresson), second and third instar of DBM larvae were about equally susceptible to parasitism by this species, and the fourth instar was less susceptible than the second and third instars (Putnam 1968). In contrast, Fox *et al.* (1990) reported that female *D. insulare* had clear oviposition preferences among DBM instars and no parasitoids emerged from DBM first instars that were offered to ovipositing wasps.

Discrimination against certain host stages may cause differences in the levels of observed parasitism. Discrimination may occur against old or young host stages, depending on the rates of encounter. However, *D. semiclausum* avoids patches of heavily-parasitised hosts and preferentially oviposits in unparasitised larvae (Legaspi 1986).

No investigation on immunological defences of DBM has been reported. However, both physical and immunological defence reactions are common in older host stages (e.g., Lewis and Vinson 1971; Taylor 1988; Allen 1990). Therefore, probably physical defence by

DBM 4th instar larvae could be at least one of the related factors responsible for the observed differential parasitism of host stages by *D. semiclausum*. Furthermore, Weisser (1994) suggested that the eggs distribution among instar classes depends on the interplay between parasitoid searching behaviour, host defence reactions, and the female's tendency to attack particular instars.

Dissection of the larvae at the end of experiments on larval instar preferences makes known only the result of the interaction between parasitoid searching behaviour, parasitoid assessment of host quality, and host behaviour (Weisser 1994). Although some studies demonstrated that dissection was superior to the rearing method for the accurate measurement of parasite and disease incidence (Day 1994), in this study the results of the rearing of hosts compared with the results of dissecting method showed no evidence for physiological defences of larval instars against parasitoids.

CHAPTER 7

PARASITISM BY *DIADEGMA SEMICLAUSUM:* EFFECTS OF HOST PLANTS (CABBAGE, WILD RADISH, AND WILD MUSTARD)

7.1. Introduction

Plant characteristics affect natural-enemy action (for an overview see: Bellows *et al.* 1992). Receiving stimuli from host plants play the primary role in attracting parasitoids to them (Monteith 1955). However, parasitoids may use chemical stimuli from their host or from the food of the host in foraging for host (Vinson 1976; Nordlund *et al.* 1981). The quality or proportion of chemical constituents of the food used by the host determines apparently the extent of successful parasitisation (Flanders 1942). Furthermore, plant architecture including "(a) plant size or surface area, (b) the variation among plant parts (structural heterogeneity), such as seed heads, flowers and nectaries, and leaves with heterogeneous surfaces (e.g., glabrous, hirsute), and (c) the connectivity of parts or plant form (structural complexity)" can influence parasitism rate (Andow and Prokrym 1990). For example, parasitism of *Pieris rapae* (L.) populations on flat, open leafed *Brassica oleracea* L. was greater than on heading or curly-leafed varieties (Pimentel 1961c). Natural enemy species may attack polyphagous herbivores on different plant species, both in crops and uncultivated plants (for an overview see: Bellows *et al.* 1992). In this study the level of parasitism was investigated on different brassicaceous plants including a crop, cabbage (*Brassica oleracea* L. cv. 'green coronet') and two weeds, wild radish (*Raphanus raphanistrum* L.) and wild mustard (*Sysimbrium orientale* L.). These weeds are the dominant brassicaceous weeds in South Australia. These host plants vary significantly in size and morphology.

7.2. Materials and methods

7.2.1. Rearing parasitoids

The parasitoids used in the experiments were reared as described in Chapter 6.

7.2.2. Host plants

Seeds of the wild hosts used in these studies were taken from weeds at Lenswood, the Northern Adelaide Hills and Virginia, South Australia (Chapter 4). For uniformity of culture, cabbage and weeds were grown in a glasshouse. Seeds of weeds were sown in plastic trays (40 cm length × 30 cm width × 11 cm depth). Seeds germinated in 5-6 days at about 22±2°C. When the seedlings were ca. 10 cm tall (usually within 12-15 days after germination), they were transplanted. The most uniform seedlings from each tray were chosen, then were transferred to the pots (12.5 cm × 12 cm), one plant per pot. Each pot was filled with UC soil. The plants were fed by dissolving 15-30 g of feeder fertilizer (36N-11P-12K) in 10 l of water and periodically using this solution to water the pots. Three plants of each species were selected for morphological similarity and were approximately equivalent in maturity. All plants were 45-days old with an average height of 24 cm and received the same treatment to maintain in the best possible condition. Plant age was based on age from sowing to date of first use in an experiment. All three hosts, cabbage, wild radish and wild mustard, were at the seven-eight leaf stage. During the test, the larvae and plants were kept in a constant environment room, maintained at 24±1°C. The photoperiod was 14L:10D.

7.2.3. Parasitism on host plants

The third instar host was selected since the parasitism of 3rd instar is a representative of all instars and this is one of the stages preferred by *D. semiclausum* and it provides a body size large enough to be dissected after being parasitised. To provide 3rd instars, newly hatched first instars were reared in cylindrical glass tubes as described in Chapter 6. Then the first instar larvae from each host plant disk in the tube were transferred to the same host plant and reared to the third instar. After third instars were available, they were collected and introduced individually on each of the host plants in each cage. One night before the start of the experiment, the 20 third instar larvae were colonised on each host plant to produce chemical cues for parasitoid activity. Levels of parasitism were determined by caging two 5 day-old female parasitoids and three potted plants with twenty DBM larvae on each plant in each cage (60 cm × 60 cm × 60 cm) (Chapter 6). For each replicate, one potted cabbage, one wild radish, and one wild mustard plant were randomly placed at a distance about 30 cm from each other in each cage. Three cages each containing female parasitoids and larvae were used. Wasps were well fed with honey and water. All female parasitoids were

released into the cages in the morning on the day of testing at 9 am. After 24 hr, the host larvae were kept at–20°C until dissected. The larval dissection procedure was the same as that described for choice experiments (Chapter 6).

7.2.4. Analysis of data

All data were transferred to percentage parasitism and analysed by ANOVA using Statistix 4.0 (Statistix User's Manual, 1992). The means were compared using Tukey HSD Pairwise Comparisons at P=0.05.

7.3. Results

7.3.1. Third larval instar parasitism on host plants

Parasitism of hosts on cabbage was higher than on the other host plants (P= 0.002) (Figure 7.1). No significant difference was observed in the rate of parasitism between wild radish and wild mustard. Thus, the number of larvae parasitised was influenced by the plant. Parasitism was 1.3 and 1.5 times higher on cabbage than on wild radish and wild mustard respectively (Figure 7.1; Table 7.1).

7.4. Discussion

Parasitoids may not parasitise a preferred host larva on an unpreferred plant species (e.g., Vinson 1981). Vinson (1976) pointed out that "a host may have a wide range of plants on which it develops, but the parasitoid may only respond to a certain number of these plants". However, there is "the possibility that the host plant may confer on the host insect a kind of immunity to parasitisation" (Smith 1942). Abortive development of the parasite, induced by the food plant of the host insect may be the cause of differences in parasitisation of an insect species on different host plants (Flanders 1942). However, if olfactory and/or chemotactile stimuli from one host plant species are more attractive to parasitoid females than similar stimuli from other host plants, more females would search for the host on the preferred host plant (Arthur 1962). Therefore, plants lacking certain chemicals may be less attractive resulting in poor parasitism (Vinson 1976; Nordlund and Sauls 1981). Furthermore, host plant structural complexity could be a significant factor influencing the dynamics of parasitoid-host systems (Andow and Prokrym 1990). In this study parasitoids

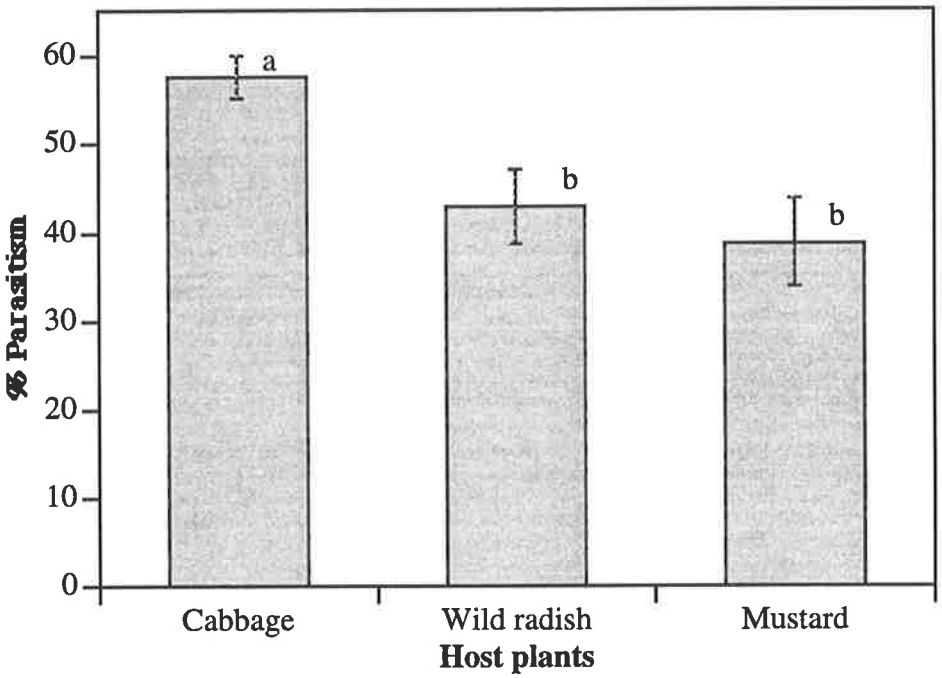


Figure 7.1. Parasitism of third instar of DBM larvae by *D. semiclausum*. The same letter at the top of each bar indicates no significant difference (P=0.05, Tukey HSD).

Table 7.1. Analysis of variance for parasitism of third instar of DBM larvae by *D. semiclausum.*

Source of variation	Degrees of freedom	Mean squares
Replication	2	36.10
Host plants	2	296.38**
Residual	4	6.78

** Significant at p <0.01.

seemed to be associated with some brassicaceous plants (crops) more than others (weeds). The results indicated that parasitism by *D. semiclausum* was considerably higher on cabbage than on wild radish or wild mustard. Thus, the rate of parasitism by *D. semiclausum* varied among crucifers. These differences might be due to different architectures or differences in the kind and amount of chemical cues released by different plant species. Parasitoids and crops associations have been reported for a numbers of herbivores (e.g., Nettles 1980; Altieri *et al.* 1981; Vinson 1984a; Okuda and Yeargan 1988; Kauffman and Kennedy 1989; van den Berg *et al.* 1990). In contrast, some workers have reported that host plants have no effect on orientation by parasitoids (Boling and Pitre 1971; Esmaili and Wilde 1971). In this study a definite preference for parasitising DBM on cabbage has been demonstrated, and presumably differences among other untested species also occur. This is in accordance with other studies which showed that different host plants can contribute to significantly different levels of parasitism of DBM by *D. semiclausum* (Beck and Cameron 1990; Talekar and Yang 1991).

CHAPTER 8

OVIPOSION BY DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) ON DIFFERENT HOST PLANTS

8.1. Introduction

The physiological condition, chemical and physical properties, climate, spatial and temporal availability of alternative hosts, and the associated flora and fauna are factors that influence colonisation and utilisation of a host plant by its herbivores (Bach 1981; Fox and Morrow 1981; Ahmad 1983; Gould and Stinner 1984; Letourneau and Fox 1989). Many factors play a role in the process of oviposition by different insects. It is likely that volatile compounds in the tissues of the host plant that can act as either deterrents or attractants, or indeed as oviposition stimulants, are the major cues used by an insect to assess the quality of a particular host plant (Leather 1994). For example, coumarin and rutin are known as ovipositional deterrents to DBM in cabbage tissue (Tabashnik 1985). Furthermore, differences in the allelochemic environment may significantly change the oviposition rate on a single host plant cultivar (Verkerk and Wright 1994). Volatile stimuli in females approach to a host plant, and contact stimuli for the final acceptance or rejection of the plant are important respectively (Anderson and Lofqvist 1996). However, plant chemistry as well as physical aspects of plant form and structure are the major determinants of host quality and this factor mediates the responses of female insect (Leather 1994). Maternal (larval and adult conditioning) and genetic factors can cause females to oviposit preferentially on the same plant species on which they fed as larvae (Rausher 1983b). The main attributes of the oviposition site are the quality and quantity of food that affect survival of the progeny (Mappes and Mäkelä 1993). However, the quality of food may be estimated by the ovipositing female on the basis of the actual food resources at the time of oviposition or according to some stimulus that is normally correlated with an adequate future supply of food during development of the progeny (Wiklund 1977). The choice of host plant by females in many phytophagous insect species, is vital for the survival of their offspring (Anderson and Lofqvist 1996). Plant characteristics associated with performance, e.g., survival of immature herbivores, that can be discriminated by

ovipositing females gives them a selective advantage over females that are not able to do so (Minkenberg and Ottenheim 1990). However, all of the complex elements of characteristics of the plant influencing larval performance may not be accurately assessed by ovipositing females (Craig *et al.* 1989). Plant attributes may affect ovipositional preference and larval performance differently (Horner and Abrahamson 1992). Nutritional quality and natural product defensive chemistry are major determinants of plant tissue suitability to phytophagous insects (Cates *et al.* 1987). Plant nitrogen content, water content, biomass and/or secondary chemistry may affect preference by herbivores for specific plants (Rosenthal and Janzen 1979; Scriber and Slansky 1981; Jansson and Smilowvitz 1986). Quality of food may depend on defensive/attractive chemicals (May and Ahmad 1983), age of the host (Lewis 1984), or nutrients (Myers 1985). For example, *Pieris rapae* (L.) showed varying ovipositional preferences for *Brassica* plants grown under different water and nutrient regimes (Wolfson 1980; Myers 1985). The cues selected from among potential host plants by ovipositing females may not be the actual determinants of larval performance. Different quantities of glucosinolates and other secondary plant chemicals in different stages of host plants have been shown to influence oviposition (Gupta and Thorsteinson 1960b; Renwick and Raadke 1990). Although plant chemicals as well as physical factors are equally important in host finding and acceptance for oviposition and/or feeding (Panda and Khush 1995), plant phenology also affects insect oviposition strategies. For example, for leaf miners the age of the leaf and nutritional status are both important (Leather 1994). Host plant density may also affect oviposition behaviour (Rausher 1983a). Oviposition preference does not necessarily indicate larval performance. However, the conditions under which plants grow might alter both the acceptability of these plants for adult insects and the suitability of these plants for their offspring, and might thus complicate comparison of preference and performance on different host species (Minkenberg and Ottenheim 1990). Not just suitability of a host plant, but its attractiveness to the reproducing female insect affect a number of decisions that must be taken once it has arrived at its new host (Leather 1994). The evolution of patterns of food plant use by herbivorous insect, is explained by chemical or morphological properties of plants (Fox and Eisenbach 1992). However, DBM oviposition is influenced by many factors including genetic factors, the climatic conditions, mating, and the presence or absence of host plants on which to oviposit (Salinas 1986) and also the plant properties

such as nutritional status (Salinas 1986; Fox and Eisenbach 1992), secondary compounds (Fox and Eisenbach 1992), as well as age of host plants (Verkerk and Wright 1994).

DBM feeds on plants in the family Brassicaceae. Cultivated species of genus *Brassica*, and weeds like wild radish, *Raphanus raphanistrum* L., and wild mustard, *Sisymbrium orientale* L. are the most common species of hosts in South Australia. The objective of this study was to determine whether DBM shows differential oviposition on potential host plants including brassicaceous crops and weeds.

8.2. Materials and methods

8.2.1. Insect collection

DBM adults, were obtained from a culture of DBM. The culture had been maintained in the laboratory for about six years at 25±1°C on cabbage. Preconditioning the test insects on particular plants prior to testing is likely to affect the oviposition of DBM behavior. In order to prevent the effect of conditioning upon emergence, DBM cocoons were collected and held in a cage separately away from the host plants. Adults of both sexes that emerged in the cocoon cage were fed honey solution and water to maitain normal activity.

8.2.2. Oviposition preference assays

A choice test was carried out to determine if the moth prefers rapeseed, wild radish or wild mustard. The selection of the two latter weeds was based on their prevalence as alternative host plants in South Australia (Chapter 4). The plants were grown from seeds in pots (12.5 cm in diam. × 12 cm in h.) in a glasshouse. After germination (as described in Chapter 7), one plant was transplanted and maintained in each pot. Experiments were conducted when the rapeseed and weed plants had 5 and 8 leaves respectively, and the height of rapeseed, wild radish, and wild mustard were 22, 24, and 25 cm respectively. Six plants, 2 of each species were randomly placed in a muslin-screened wooden cage (60 cm × 60 cm ×60 cm) (Chapter 6). In a second experiment, six plants, 3 of each wild radish and wild mustard, were placed in a cage. Twenty one day-old mated female adults were released into each cage. Factors like light and temperature (28°C) were approximately similar within cages. After 24 hr, plants were removed and the numbers of eggs deposited on the leaves and/or

pot walls and net were counted. This experiment was replicated by repeating it at three times.

8.2.3. Statistical analysis

The experiment was analysed as a Randomised Complete Block. Therefore, effect of host plant on oviposition was determined by analysis of variance (ANOVA) using the General Linear Models procedure(GLM) of SAS (SAS Institute 1993).

8.3. Results

8.3.1. Oviposition preference

There was no detectable preference for any of the three plants tested. Day to day variation in the results was high. For example, rapeseed was ranked 1st in one replicate and last in another. In the treatments in which the crop plants were present, oviposition was greatest on the rapeseed. However, there was no significant difference in numbers of eggs laid on either weeds whether or not crop plants were present in the cages (Figure 8.1; Table 8.1).

DBM was observed to oviposit on leaves of all ages. On all host plants the eggs were found mostly on and along the veins of the leaves. The distribution of eggs was the same as described by Uematsu and Sakanoshita (1989) on Japanese radish (*Raphanus sativus* L., cultivar: Sai). In a few cases a few eggs were deposited on the pots; these eggs were recorded as well.

8.4. Discussion

Oviposition is not an accidental act (Panda and Khush 1995), but may be influenced by the physiological state of the insect or the state of the environment (Courtney and Kibota 1990; Jaenike 1990; Bernays and Chapman 1994). Clutch size is influenced by a number of factors: the frequency of ovi- or larviposition, the size of the mother, the number of eggs/offspring produced, and most importantly, the quality of the host. Clutches are small and are produced at a slower rate on poor quality hosts than on good quality hosts (Leather 1994). In this study, oviposition by DBM was not influenced by the host plants rapeseed, wild radish and wild mustard. However, Fox and Eisenbach (1992) in a study of

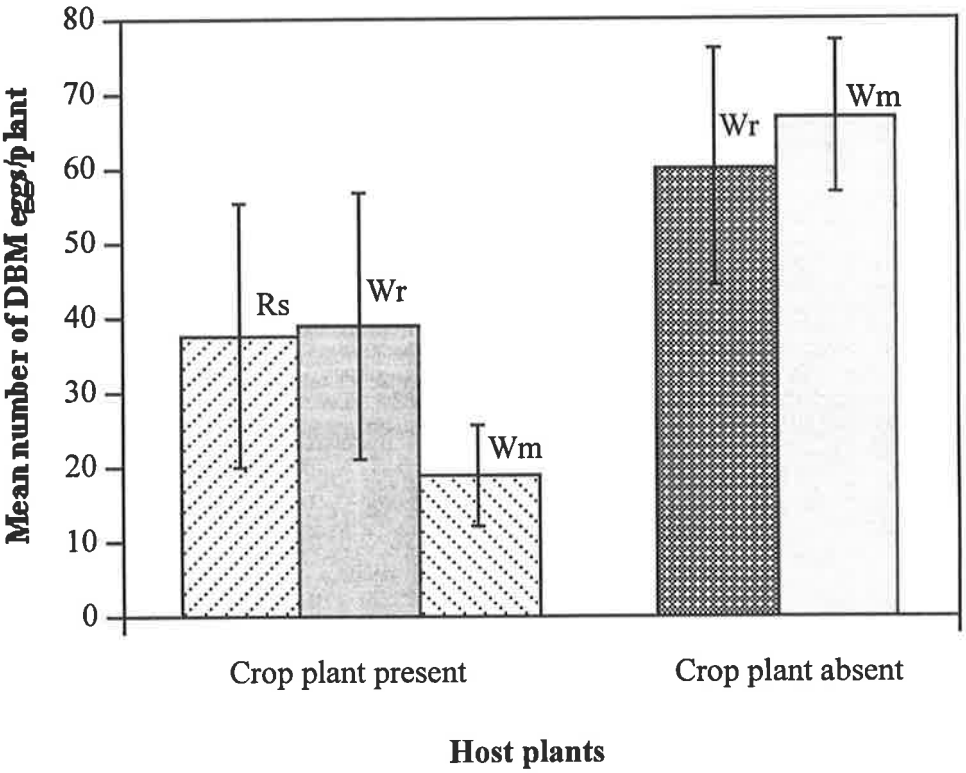


Figure 8.1. Ovipositional preferences of DBM on wild radish and wild mustard with and without the presence of crop plant (rapeseed). Values are mean ± SE. Rs, Rapeseed; Wr, Wild radish; Wm, Wild mustard

Table 8.1. Analysis of variance for ovipositional preferences of DBM on wild radish and wild mustard with (a) and without (b) the presence of crop plant (rapeseed).

a) With the presence of crop plant.

Source of variation	Degrees of freedom	Mean squares
Day	2	5345.17**
Plant species	2	743.17
Residual	13	731.79

** Significant at $p < 0.01$.

b) Without the presence of crop plant.

Source of variation	Degrees of freedom	Mean squares
Day	2	508.67
Plant species	1	213.56
Residual	14	1778.22

preferences on five crucifers including three wild species and two varieties of *Brassica oleracea* L. (cole crops), showed that both the DBM and its main parasitoid, *Diadegma insulare* Cresson preferred all wild crucifers, over most (DBM) or all (wasp) crop varieties.

In the experiment reported here, DBM was shown to oviposit on all three of the brassicaceous plants that occur in South Australia. It can be expected that moth will accept the weeds as hosts, and DBM was shown to feed on these weeds in the field (Chapter 4). It should be noted that the behaviour of DBM may differ if the moths are not caged since cages short-circuit long-distance orientation, and close proximity of plants may interfere with discrimination.

CHAPTER 9

SUITABILITY OF DIFFERENT HOST PLANTS FOR DIAMONDBACK MOTH

9.1. Introduction

The utilization of a host plant by an herbivorous insect involves at least two steps. These are the recognition and acceptance of a plant by an ovipositing or feeding individual, and the suitability for the performance (growth, survival and fecundity) of the feeding individual (Horner and Abrahamson 1992).

A number of plant characteristics can affect the suitability of a host plant for larval survival and growth. For example, water content (Scriber 1977, 1984), chemical defences (e.g., Rosenthal and Janzen 1979), the amounts of nutrients such as nitrogen (e.g., Mattson 1980; Scriber 1984), and probably most frequently a combination of these attributes (Horner and Abrahamson 1992) are known to affect insect growth and survival. Exploitation by herbivores may be restricted by some patterns of plant phenology (Barbosa 1988), and consequently seasonal occurrence may be another defense mechanism (Yano and Ohsaki 1993). Defense mechanisms of plants such as tough tissues, dense trichomes and chemical toxins are responsible for their low intrinsic quality as food, and plants have evolved such mechanisms against herbivorous insects and other organisms (Yano and Ohsaki 1993). In brassicaceous plants, mustard glucosinolates are known to be qualitative defences (for an overview see: Yano and Ohsaki 1993). Some herbivores utilize the defensive chemicals as a cue for their feeding when they have defeated such an obstacle (David and Gardiner 1966) and become specialised on certain plants (Yano and Ohsaki 1993).

Diamondback moth has adapted to host plants in the family Brassicaceae (e.g., Harcourt 1986; Talekar and Shelton 1993; Muhamad *et al.* 1994). Favoured cultivated crops, and weedy plants serve as alternative hosts (e.g., Talekar and Shelton 1993; Muhamad *et al.* 1994). The objective of this study was to investigate the developmental time and survival of larval DBM placed on cabbage, wild radish, and wild mustard as these are the most prevalent alternative host plants of DBM in South Australia.

9.2. Materials and methods

9.2.1. Experimental design

Cabbage, wild radish, and wild mustard were grown in a glasshouse as described in Chapter 7. Wild radish and wild mustard had 8 leaves and their mean height was 22 cm, whereas cabbage plants had 7 leaves and were 20 cm tall. During the experiment the plants grew 10-15 cm. Six host plants of a given species were placed in one cage (60 cm × 60 cm ×60 cm) (Chapter 6), and there were two cages for each species. Eggs on each host plant were obtained by holding moths in a cylindrical glass tube with discs of leaf tissue from each of the plant species (Chapter 6). Immediately upon hatching, first instar larvae from each host plant disc were transferred with a fine camel hair brush in groups of ten to each potted plant. These larvae had not previously fed upon any plant material. In this experiment, development was followed up to adult emergence. Newly emerged adults were counted and removed daily. Survivorship was calculated as the fraction of the initial 60 larvae in each cage that emerged as adults at the end of each experiment. This experiment was carried out in a growth chamber at 24°C with a 14L:10D photoperiod. Lighting was provided by overhead fluorescent lights. The experiment was replicated 4 times.

9.2.2. Analysis of data

Data were analysed with analysis of variance (ANOVA) using a Randomized Complete Block Design. Differences in the developmental times of DBM on different host plants were determined by the General Linear Models (GLM) procedure and pairwise contrasts of least squares means of SAS (SAS Institute 1993). Percentage adult emergence was arcsine transformed prior to analysis of variance to stabilise variances. Since the trials showed no difference in survival on all plants, the power of ANOVA (1-β) was detemined (Zar 1984).

9.3. Results

Larvae developed on cabbage, wild radish, and wild mustard. DBM larval survival was the same on all plants but the developmental period of immature stages was significantly longer on wild radish (P <0.05) (Tables 9.1 - 9.2). The estimated power of the ANOVA used to test for differences in survivorship was 0.95. This is the probability of rejecting an

incorrect null hypothesis of no difference in survival between host plant species given a 10% difference in larval survival among host plants.

Table 9.1. Mean percentage DBM adults emerged from larvae (survival) and developmental time larval DBM reared on cabbage, wild radish and wild mustard. Values are least squares mean ± SE. Values followed by the same letter in the second row are not significantly different as determined by pairwise comparisons of least squares means. A total of 480 larvae were released at the start of the experiment (120/rep. × 4 replicates).

	Cabbage	Wild radish	Wild mustard
Survival (%)	85±4.9	82.5±5.4	78.5±4.9
Developmental time (day)	14.1±0.1a	14.7±0.1b	14.2±0.1a

9.4. Discussion

Wild radish and wild mustard as well as cabbage are important host plants of the DBM in South Australia (Chapter 4). These weeds are locally abundant in vegetable growing areas and serve as alternative host plants for DBM. The development of DBM on the three brassicaceous plants, indicated that the DBM is adapted to all of these host plants. Furthermore, this study showed that the development of larvae fed on three plant species was significantly different. However, in a report by Yamada (1983, cited by Shirai 1993) the developmental periods of larval DBM reared on the fresh leaves of four cruciferous weeds (*Capsella bursa-pastoris, Cardamine flexuosa, Rorippa palustris* and *R. indica*) were not significantly different from those reared on cultivated radish and cabbage leaves. Caution must be used in interpreting Yamada's results as excised leaves may not have the same nutritional value as intact plants. Under the circumstances chosen in the laboratory experiments, no significant difference was found in survival rate on the selected species (Table 9.1). It seems that the intrinsic quality of the tested plants as food for larval DBM was similar.

It should be noted that the nutritional quality of plants depends on the availability of nutrients, e.g., nitrogen (Scriber and Slansky 1981). Crops and weeds growing in cultivated fields may have a high nutritional value following the application of fertilizers.

Table 9.2. Analysis of variance for survival (a) and developmental time (b) of larval DBM reared on cabbage, wild radish and wild mustard.

a) Larval survival

Source of variation	Degrees of freedom	Mean squares
Replication	3	0.o84**
Plant species	2	0.0084
Residual	6	0.0111

** Significant at p <0.01.

b) Developmental time

Source of variation	Degrees of freedom	Mean squares
Replication	3	309.77***
Plant species	2	43.73***
Residual	6	1.38

*** Significant at p <0.001

Conversely weeds on uncultivated land may be less nutritious. Thus although there were no differences detected in survival among the plant species, it is likely that edaphic factors will influence the suitability of these plants in the field.

The developmental time was significantly shorter on cabbage and wild mustard than on wild radish indicating that cabbage and wild mustard are nutritionally better hosts. Furthermore, the differences in developmental times between host plant species could be important in the context of host-parasitoid dynamics over a number of generations. Larvae that are present on plants for longer periods will be susceptible to greater levels of predation and parasitism. Hence, the longer developmental time on wild radish should lead to a higher mortality on this host plant than other plants.

CHAPTER 10

SUMMARY AND CONCLUSIONS

A study of population dynamics in predator-prey or parasite-host systems provides knowledge of the potential value of natural enemies (Waage 1992). Given the limited knowledge of diamondback moth (DBM) and its adverse economic impact in South Australia (SA), it is essential to gain a better understanding of its population dynamics. The annual pattern of abundance is generated by factors such as availability of food, predation, competition, and abiotic factors that operate at the individual level on insect herbivores (Haukioja 1993). Therefore, the main goals of this project were to quantify populations of DBM and its parasitoids, particularly *Diadegma semiclausum* Hellen on brassicaceous crops and spring weeds, in order to gain a more comprehensive knowledge of the dynamics of this pest (Chapters 3-4). In this final chapter of the thesis experimental results are summarised and integrated.

The pest status of DBM is due both to its potential to cause severe damage to a wide range of brassicaceous vegetables (e.g., Harcourt 1954; Talekar *et al.* 1985, 1986; Waterhouse and Norris 1987) and to its resistance to many commonly used insecticides (e.g., Talekar *et al.* 1985, 1986; Tabashnik 1986). In addition the mobility and short generation time of DBM, and the changing availability of food sources as influenced by plant phenology lead to great variation of DBM density throughout the year (Pittendrigh and Pivnick 1993). The serious damage caused by DBM in brassicaceous crops in Australia in the late 19th and early 20th centuries (Waterhouse and Norris 1987) on one hand, and its resistance to different classes of insecticides (Altmann 1988) on the other, has resulted in an increased demand for alternative methods to control this pest.

The pattern of appearance and infestation of DBM in the Adelaide region of South Australia revealed that DBM often occurred in high numbers on brassicaceous weeds in field boundaries, roadside verges and other unploughed areas in early spring. These DBM exploit wild hosts like wild radish and wild mustards for a restricted period each year, usually from autumn to late spring according to climatic conditions. After weeds senesce in late spring, large numbers of DBM probably move onto crops. New weeds which

germinate with the first soaking rainfall in autumn, appear to provide important refuges for the DBM that disperse from brassicaceous crops at the end of summer. This alternation of host plants may retard the evolution of resistance to insecticides. This is due largely to the mixing of insecticide-resistant populations with populations free from this selection pressure in weed verges not normally treated with insecticides and the invasion of new crops with susceptible individuals. In addition parasitoids of DBM may spread from crop fields to weeds and vice versa. Thus, frugal use of insecticides, in combination with pathogens, natural enemies, and cultural controls is considered the most promising approach for delaying insecticide resistance in DBM (Tabashnik and Mau 1986). To spray populations of DBM only during alternating generations essentially increases the time taken for the evolution of resistance. This could be achieved by spraying crops at critical population levels rather than, as in current practice of routine spraying, often, several times per week.

DBM was shown to be regularly parasitised by three larval and two pupal parasitoids in South Australia. Parasitism varies from year to year. The greatest level of parasitism occurs in the period from October to January each year. A number of techniques were investigated for measuring the impact of parasitoids on DBM. Each method was shown to have limitations, and hence no single method alone will provide complete information on levels of parasitism. Conventional density samples provide information on the density of DBM but give biased estimates of parasitism. Restricting focus to the 4th instars did not appear to improve the estimates, however, the number of parasitised fourth instars may represent a reasonable reflection of a seasonality pattern of DBM parasitism by *Diadegma* species. A recruitment method gave different results, and larval mortality prior to pupation led to biased estimates of host recruitment. Thus, one must use a combination of methods to obtain an understanding of the role of parasitoids in the dynamics of DBM. When the data were considered overall, larval parasitism was shown to be low and variable on all host plants and, second to "unexplained mortality" (see Chapters 3-4), appeared to be a major mortality factor for larval DBM. *D. semiclausum* was found to be the dominant parasitoid of DBM. There was not a consistent relationship between rate of parasitism and host density (Chapters 3-4). In addition, egg parasitoids and predators probably also accounted for a proportion of the deaths of DBM larvae. Predators like syrphid larvae and ants were often observed during this study.

Knowledge of factors that determine the distribution of pest and natural enemy species within a field can be used to target more precisely control activities or other forms of population manipulation both in time and space (Kozar *et al.* 1994). Apart from natural enemies, a major determinant of the diversity of DBM is the seasonal development of the host plants. Plants grow more vigorously in spring and food plants are abundant. This is consistent from year to year. Clearly, the crops that were planted and methods that used to manage them can affect the presence of DBM. Of the brassicaceous weeds, wild radish and wild mustards were abundant in winter and spring. Population levels of DBM were found to vary greatly among years on them. Climatic conditions and weather, day length, temperature, and rainfall are changing the availability of food plants. Brassicaceous weeds exhibit a reduction in leaf quality at the end of spring and completely dry off in summer. DBM depends on these plants as seasonal resources. When there is a marked deterioration of food plant availability or quality, local dispersal to more favourable areas allows them to survive when food becomes limiting. Therefore, DBM adults which are present throughout the year appear to oviposit adventitiously on any available brassicaceous host plants. Since reproductive activity of DBM declines during summer due to high temperatures and plant senescence, DBM must be sufficiently flexible and robust to accommodate the temporal and spatial variability in food resources.

The annual activity of DBM as determined from pheromone traps confirmed that DBM does not diapause (Chapter 5). The pattern of trap catches clearly illustrated that DBM is able to survive the dry season, as well as all other seasons, and breeds throughout the year by selecting favourable habitats. However, there is still little information on the mechanisms of long-distance migration.

In order to best counter the damaging effect of insect pests, a knowledge of the activity of parasitoids and predators, that limit pest populations is necessary (Mustata 1992). Laboratory experiments were undertaken to determine which instar *D. semiclausum* prefers. It was found that even though this parasitoid exploits all instars, percentage parasitism of the 2nd instar was higher than all others (Figures 6.1-6.2). Percentage parasitism of the third instar was investigated on different host plants. Parasitism was higher on cabbage compared to wild radish and wild mustard (Figure 7.1). Oviposition by

DBM on wild radish, wild mustard and rapeseed was equal (Figure 8.1), and the developmental time was similar on cabbage, and wild mustard, but one half day longer on the wild radish at 24 °C (Table 9.1). There was no statistical difference between larval survival on all three host plants (Table 9.1). It is likely that weeds growing in uncultivated areas are less nutritious since they are not fertilised. Hence development is probably slower on such weeds. Generally results indicated that DBM has adapted to brassicaceous host plants and this enables it to increase rapidly in numbers when host plants are available. As parasitism is somewhat lower on weeds than crops, weeds are probably a major source of DBM that move into crops in spring.

Despite the reported tolerance of DBM to many crop insecticides, there have been no reports relating to the tolerance of *D. semiclausum* to insecticides. Consequently, to maximize effectiveness of parasitoids fully in the management of DBM in vegetable farms, growers must minimize the effects of insecticides on parasitoids. Basic to this goal is the development of an Integrated Pest Management (IPM) program. Fundamental to the development of a comprehensive pest management system for DBM, in the hills and horticultural areas in SA, is an understanding of its seasonal dynamics.

Considerable variation among sites was found in the magnitude and timing of the peak densities of DBM feeding on weeds. Patterns of parasitism varied geographically in a similar manner (Chapter 4). The populations of DBM on weeds are potential invaders of adjacent crops. Therefore, the observed temporal and spatial variation in the size of populations of DBM and the associated parasitoids indicate that IPM for DBM needs a local, rather than regional, focus.

This thesis investigated a complex of natural enemies that parasitises DBM in SA (Chapters 3-4). The major parasitoid, *D. semiclausum* together with two other parasitoids *Diadegma rapi* (Cameron), and *Apanteles ippeus* Nixon, attack the larval stages of the DBM while *Brachymeria phya* (Walker) and *Diadromus collaris* (Gravenhorst) attack the pupae. Although the future of parasitoids in pest control will very much depend upon their compatibility with pesticide use (Waage and Hassell 1982), it is possible that *D. semiclausum* could become more important in an IPM program in the future. The present results indicate that, despite the low rate of parasitism by *D. semiclausum* and *D. rapi* in

SA, these parasitoids play an important role in the dynamics of DBM in the field. The parasitoids were relatively abundant in each year of sampling. To better exploit parasitoids, insecticide rates should be reduced and insecticides should be selected that are least harmful to the parasitoids, but still relatively toxic to DBM. By urging vegetable producers to follow this recommendation, conservation of native and introduced parasitoids should be better achieved, resulting in a greater, and possibly more consistent level of DBM biological control in vegetable farms. However, little is known of the effect of microbial control and the impact of predators on DBM in SA. Disease, availability of food, weather, migration, and use of insecticides all play a vital role in the course of DBM populations, yet the relative contribution of its parasitoids in population control should not be underestimated.

Appendix 1. Total numbers of DBM emerged and percent parasitism by *Diadegma semiclausum* during a field survey on rapeseed and kale plants at Lenswood, South Australia.

September 1993 to January 1994

Sample date	Sample size (quadrats)	2nd instar emerged	Para-sitised	% para-sitism	3rd instar emerged	Para-sitised	% para-sitism	4th instar emerged	Para-sitised	% para-sitism
7-Sept-1993	15	0	0	**0**	0	0	**0**	0	0	**0**
14-Sep	15	5	0	**0**	0	0	**0**	2	0	**0**
27-Sep	10	6	0	**0**	4	0	**0**	1	0	**0**
7-Oct	10	5	0	**0**	6	0	**0**	4	0	**0**
15-Oct	10	7	0	**0**	3	0	**0**	3	0	**0**
22-Oct	10	5	0	**0**	15	0	**0**	5	0	**0**
30-Oct	5	2	0	**0**	0	0	**0**	5	0	**0**
7-Nov	15	24	2	**8.3**	14	0	**0**	28	4	**14.3**
16-Nov	15	49	2	**4.1**	49	4	**8.2**	40	2	**5.0**
23-Nov	10	58	0	**0**	51	3	**5.9**	63	3	**4.8**
30-Nov	10	17	0	**0**	123	13	**10.6**	193	14	**7.3**
6-Dec	10	7	0	**0**	39	1	**2.6**	140	40	**28.6**
20-Dec	10	33	5	**15.2**	50	13	**26.0**	21	10	**47.6**
30-Dec	10	10	2	**20.0**	14	3	**21.4**	35	17	**48.6**
11-Jan-1994	10	2	2	**100.0**	1	1	**100.0**	4	2	**50.0**
18-Jan	10	0	0	**0**	0	0	**0**	9	7	**77.8**
25-Jan	10	0	0	**0**	0	0	**0**	2	0	**0**
31-Jan	10	0	0	**0**	0	0	**0**	0	0	**0**

Appendix 2. Total numbers of DBM emerged and percent parasitism by *Diadegma species* during a field survey on kale at Lenswood, South Australia.

October 1994 to January 1995

Sample date	Sample size (quad-rats)	2nd instar emerged	Para-sitised (*Ds*)	% para-sitism (*Ds*)	Para-sitised (*Dr*)	% para-sitism (*Dr*)	3rd instar emerged	Para-sitised (*Ds*)	% para-sitism (*Ds*)	Para-sitised (*Dr*)	% para-sitism (*Dr*)	4th instar emerged	Para-sitised (*Ds*)	% para-sitism (*Ds*)	Para-sitised (*Dr*)	% para-sitism (*Dr*)
24-Oct-1994	5	2	0	**0.0**	0	**0.0**	0	0	**0.0**	0	**0.0**	4	0	**0.0**	0	**0.0**
31-Oct	5	6	0	**0.0**	0	**0.0**	3	0	**0.0**	0	**0.0**	5	0	**0.0**	0	**0.0**
4-Nov	10	66	1	**1.5**	4	**6.1**	85	10	**11.8**	2	**2.4**	76	6	**7.9**	2	**2.6**
10-Nov	5	9	0	**0.0**	0	**0.0**	28	0	**0.0**	0	**0.0**	32	1	**3.1**	0	**0.0**
14-Nov	5	34	3	**8.8**	0	**0.0**	19	3	**15.8**	0	**0.0**	47	4	**8.5**	1	**2.1**
22-Nov	5	6	2	**33.3**	1	**16.7**	1	1	**100.0**	0	**0.0**	10	5	**50.0**	0	**0.0**
28-Nov	10	76	13	**17.1**	0	**0.0**	39	4	**10.3**	3	**7.7**	79	12	**15.2**	7	**8.9**
6-Dec	10	45	1	**2.2**	1.0	**2.2**	197	10	**5.1**	4	**2.0**	371	14	**3.8**	39	**10.5**
13-Dec	10	17	1	**5.9**	0	**0.0**	70	3	**4.3**	0	**0.0**	192	7	**3.6**	12	**6.3**
19-Dec	10	30	5	**16.7**	0	**0.0**	37	2	**5.4**	1.0	**2.7**	113	9	**8.0**	4	**3.5**
26-Dec	10	13	1	**7.7**	0	**0.0**	12	1	**8.3**	0	**0.0**	28	7	**25.0**	1	**3.6**
2-Jan-1995	10	13	3	**23.1**	2	**15.4**	19	10	**52.6**	0	**0.0**	57	29	**50.9**	11	**19.3**
9-Jan	10	34	4	**11.8**	0	**0.0**	27	10	**37.0**	1.0	**3.7**	36	6	**16.7**	3	**8.3**
16-Jan	10	7	0	**0.0**	0	**0.0**	7	0	**0.0**	0	**0.0**	23	8	**34.8**	0	**0.0**
23-Jan	10	0	0	**0.0**	0	**0.0**	1	0	**0.0**	0	**0.0**	4	0	**0.0**	0	**0.0**

Ds: *Diadegma semiclausum*; *Dr*: *Diadegma rapi*

Appendix 3. Number of DBM and parasitoids based on larvae per quadrats per week and average percentage parasitism and mortality of DBM larvae at Lenswood, South Australia.

a) rapeseed and kale 1993-94

instar	No. of larvae collected	Total no. of larvae dead*	No. of DBM emerged	No. of *Ds* emerged	No. of *Ai* emerged
2nd	471	241	200	13	17
3rd	661	292	299	38	32
4th	784	229	426	99	30
Total	1916	762	925	150	79

instar	% unexplained mortality	% emergence DBM	% parasitism *Ds*	% parasitism *Ai*
2nd	51.2	83.0	5.7	7.4
3rd	44.2	81.0	10.3	8.7
4th	29.2	76.8	17.8	5.4
Average	39.8	80.2	13.0	6.8

b) for kale 1994-95

instar	No. of larvae collected	Total no. of larvae dead*	No. of DBM emerged	No. of *Ds* emerged	No. of *Dr* emerged	No. of *Ai* emerged
2nd	540	182	270	34	8	46
3rd	740	195	415	54	11	65
4th	1420	343	777	108	80	112
Total	2700	720	1462	196	99	223

Larval instars	% unexplained mortality	% DBM emergence	% parasitism *Ds*	% parasitism *Dr*	% parasitism *Ai*
2nd	33.7	75.4	9.5	2.2	12.8
3rd	26.4	76.1	9.9	2.0	11.9
4th	24.2	72.1	10.0	7.4	10.4
Average	26.7	73.8	9.9	5.0	11.3

*Number of dead larvae in the field and laboratory during rearing by unexplained mortality factors.
Ds: *Diadegma semiclausum*; *Dr*: *Diadegma rapi*; *Ai*: *Apanteles ippeus*
Quadrat: 50 cm ×50 cm

Appendix 4. Seasonal trend and percentage parasitism of *P. xylostella* larval populations on rapeseed and kale per quadrats per week at Lenswood, SA, during the first year survey 1993-94.

Date	No. of quadrats	Total larvae/m^2	Total larvae collected	Total larvae died[1]	% mortality	Total emerged	No. of *Ds*	% parasitism *Ds*	No. of *Ai*	% parasitism *Ai*	No. of DBM	% parasitism DBM
7-Sep-1993	15	-	-	-	-	-	-	-	-	-	-	-
14-Sep-93	15	5	19	12	63.2	7	0	0.0	0	0.0	7	100.0
21-Sep-93	*	*	*	*	*	*	*	*	*	*	*	*
27-Sep	10	10	26	15	57.7	11	0	0.0	0	0.0	11	100.0
7-Oct	10	16	39	24	61.5	15	0	0.0	1	6.7	14	93.3
15-Oct	10	10	24	11	45.8	13	0	0.0	4	30.8	9	69.2
22-Oct	10	17	43	18	41.9	25	0	0.0	5	20.0	20	80.0
30-Oct	10	10	13	6	46.2	7	0	0.0	0	0.0	7	100.0
7-Nov	15	32	120	54	45.0	66	6	9.1	7	10.6	53	80.3
16-Nov	15	59	223	85	38.1	138	8	5.8	12	8.7	118	85.5
23-Nov	10	102	256	84	32.8	172	6	3.5	12	7.0	154	89.5
30-Nov	10	207	517	184	35.6	333	27	8.1	18	5.4	288	86.5
6-Dec	10	111	278	92	33.1	186	41	22.0	10	5.4	135	72.6
13-Dec	*	*	*	*	*	*	*	*	*	*	*	*
20- Dec	15	44	166	62	37.3	104	28	26.9	5	4.8	71	68.3
30-Dec	10	54	134	75	56.0	59	22	37.3	5	8.5	32	54.2
7-Jan-1994	*	*	*	*	*	*	*	*	*	*	*	*
11-Jan**	10	16	40	33	82.5	7	5	71.4	0	0.0	2	28.6
18-Jan	10	6	16	7	43.8	9	7	77.8	0	0.0	2	22.2
25-Jan	10	1	2	0	0.0	2	0	0.0	0	0.0	2	100.0
31-Jan	10	0	0	0	0.0	0	0	0.0	0	0.0	0	0.0
Total		700	1916	762		1154	150		79		925	

1: Unexplained mortality; *Ds*: *Diadegma semiclausum*; *Ai*: *Apanteles ippeus*; -: no host (DBM larvae) present; *: samples were not taken because of rainy and/or wet conditions in the field; **: after the second week in January, there were too few DBM larvae to accurately estimate percent parasitism.

Appendix 5. Seasonal trend and percentage parasitism of *P. xylostella* larval populations on kale per quadrats per week at Lenswood, SA, during the second year survey 1994-95.

Date	No. quadrats	Total larvae/m^2	Total larvae collected	Total larvae died[1]	% mortality	Total larvae emerged	No. *Ds*	% parasitism *Ds*	No. *Dr*	% parasitism *Dr*	No. *Ai*	% parasitism *Ai*	No. of DBM	% DBM emerged
18-Oct-1994	5	-	-	-	-	-	-	-	-	-	-	-	-	-
24-Oct	5	4.8	6	0	0	6	0	0	0	0	0	0	6	100
31-Oct	5	11.2	14	0	0	14	0	0	0	0	0	0	14	100
4-Nov	10	120.4	301	74	24.6	227	17	7.5	8	3.5	1	0.4	201	88.5
10-Nov	5	72.4	91	22	24.2	69	1	1.4	0	0	0	0	68	98.6
14-Nov	5	108	135	35	25.9	100	10	10	1	1	1	1	88	88
22-Nov	5	24.8	31	14	45.2	17	8	47.1	1	5.9	0	0	8	47.1
28-Nov	10	126	315	121	38.4	194	29	14.9	10	5.2	19	9.8	136	70.1
6-Dec	10	347.5	869	256	29.5	613	25	4.1	44	7.2	98	16	446	72.8
13- Dec	10	146.4	366	87	23.8	279	11	3.9	12	4.3	35	12.5	221	79.2
19-Dec	10	86.4	216	36	16.7	180	16	8.9	5	2.8	18	10	141	78.3
26-Dec	10	26	65	12	18.5	53	9	17	1	1.9	6	11.3	37	69.8
2-Jan-1995	10	48.8	122	33	27	89	42	47.2	13	14.6	13	14.6	21	23.6
9-Jan*	10	41.6	104	7	6.7	97	20	20.6	4	4.1	23	23.7	50	51.5
16-Jan	10	20	50	13	26	37	8	21.6	0	0	9	24.3	20	54.1
23-Jan	10	6	15	10	66.7	5	0	0	0	0	0	0	5	100
Total	Total		2700	720		1980	196		99		223		1462	

1: Unexplained mortality; *Ds*: *Diadegma semiclausum*; *Dr*: *Diadegma rapi*; *Ai*: *Apanteles ippeus*; -: no host (DBM larvae) present; *: after the second week in January, there were too few DBM larvae to accurately estimate percent parasitism.

Appendix 6. The phenology and general growth stage of more prevalent brassicaceous weeds in the Adelaide region, South Australia, 1993-95.

A1.1. The study plant species

A.1.1.1. *Wild radish Phenology*

Wild radish (*Raphanus raphanistrum* L.) is a common brassicaceous weed in South Australia (SA). It is an annual, erect plant growing to 1 m, and its basal leaves form a rosette. It grows in arable soils in spring and summer (Prescott 1988). It also grows in disturbed areas such as roadsides and fallow fields. While thought to be of Eurasian origin, this species is now an abundant agricultural weed on six continents (Holm *et al.* 1979). It has a broad geographic range in South Australia (Jessop and Toelken 1986).

R. raphanistrum populations are often polymorphic for flower color, displaying high frequencies of both yellow and white-flowered individuals with dark veins (Kay 1976; Wilding *et al.* 1986).

At Lenswood (Figure 3.1), wild radish was both patchily distributed and grew in large clumps up to 1000 m^2. Wild radish patches were located approximately 700 meters away from the cultivated fields (rapeseed and kale). It also occured abundantly along farm edges and roadsides verges. Mature wild radish plants are 90 to 100 cm tall. Wild radish grows flower stalks and blooms in early spring and produces seeds from late spring (mid-November) into summer. Its seeds mature and subsequently the plants wither and die back in early summer.

A.1.1.2. *Mustard Phenology*

Mustards grow in agricultural areas and disturbed soils (Prescott 1988; Wilding *et al.* 1986) and are the most common brassicaceous species in SA (Jessop and Toelken 1986). Hedge mustard (*Sisymbrium officinale* (L.) Scop.) is an erect herb up to 90 cm. In the rosette stage the leaf apex is short and rounded. In Indian hedge mustard (*Sisymbrium orientale* L.) the leaf apex is longer and pointed. Its leaves are hairy and it grows to 90 cm tall. Both *S. officinale* and *S. orientale* are annual or biennial herbs, and they have a broad geographic range in South Australia (Jessop and Toelken 1986). Giant mustard (*Rapistrum rugosum* (L.)), is an erect herb up to 80 cm tall. It is an annual or biennial herb and has a broad range of distribution in South Australia (Jessop and Toelken 1986).

Appendix 7. Number of DBM and parasitoids based on larvae per sweeping net per week and average percentage parasitism and mortality of DBM larvae in the Adelaide region, South Australia, 1994.

a) wild radish at Lenswood

Instar	No. of larvae collected	Total no. of larvae dead*	No. of DBM emerged	No. of *Ds* emerged	No. of *Dr* emerged	No. of *Ai* emerged
2nd	174	82	79	0	0	13
3rd	167	56	99	2	1	9
4th	265	48	204	3	0	10
Total	606	186	382	5	1	32

Instar	% unexplained mortality	% emergence DBM	% parasitism *Ds*	% parasitism *Dr*	% parasitism *Ai*
2nd	47.1	85.9	0.0	0.0	14.1
3rd	33.5	89.2	1.8	0.9	8.1
4th	18.1	94.0	1.4	0.0	4.6
Average	30.7	91.0	1.2	0.2	7.6

b) wild mustards in the northern Adelaide Hills

Instar	No. of larvae collected	Total no. of larvae dead*	No. of DBM emerged	No. of *Dr* emerged	No. of *Ai* emerged
2nd	743	185	452	28	78
3rd	885	223	510	26	126
4th	1871	474	1169	128	100
Total	3499	882	2131	182	304

Instar	% unexplained mortality	% emergence DBM	% parasitism *Dr*	% parasitism Ai
2nd	24.9	81.0	5.0	14.0
3rd	25.2	77.0	3.9	19.0
4th	25.3	83.7	9.2	7.2
Average	25.2	81.4	7.0	11.6

*Number of dead larvae in the field and laboratory during rearing by unexplained mortality factors.
Ds: *Diadegma semiclausum*; *Dr*: *Diadegma rapi*; *Ai*: *Apanteles ippeus*

Appendix 7 (continued)

c) wild mustards at Virginia

Instar	No. of larvae collected	Total no. of larvae dead*	No. of DBM emerged	No. of *Ds* emerged	No. of *Dr* emerged	No. of *Ai* emerged
2nd	12	3	8	0	0	1
3rd	83	16	58	3	0	6
4th	184	9	136	28	6	5
Total	279	28	202	31	6	12

Instar	% unexplained mortality	% emergence DBM	% parasitism *Ds*	% parasitism *Dr*	% parasitism *Ai*
2nd	25.0	88.9	0.0	0.0	11.1
3rd	19.3	86.6	4.5	0.0	9.0
4th	4.9	77.7	16.0	3.4	2.9
Average	10.0	80.5	12.4	2.4	4.8

*Number of dead larvae in the field and laboratory during rearing by unexplained mortality factors.
Ds: *Diadegma semiclausum*; *Dr*: *Diadegma rapi*; *Ai*: *Apanteles ippeus*

REFERENCES

Abbas, M. S. T. (1988). Biological and ecological studies on *Diadegma semiclausum* Hellen (Hym., Ichneumonidae), a larval parasite of the diamond-back-moth, *Plutella xylostella* (L.) (Lep., Plutellidae) in Egypt. *ANZ.-Schaedlingskd.-Pflanzenschutz-Umweltschutz* **61:** 1-2.

Ahmad, S. (1983). *Herbivorous insects: host-seeking behavior and mechanisms.* Academic Press, New York.

Allen, G. R. (1990). Influence of host behaviour and host size on the success of oviposition of *Cotesia urabae* and *Dolichogenidea eucalypti* (Hymenoptera: Braconidae). *Journal of Insect Behaviour* **3:** 733-749.

Alteiri, M. A., van Schoohoven, A. and Doll, J. (1977). The ecological role of weeds in insect pest management systems: a review illustrated by bean (*Phaseolus vulgaris*) cropping systems. *PANS* **23:** 195-205.

Altieri, M. A., Lewis, W. J., Nordlund, D. A., Gueldner, R. C. and Todd, J. W. (1981). Chemical interactions between plants and *Trichogramma* wasps in Georgia soybean fields. *Protection Ecology* **3:** 259-263.

Altmann, J. A. (1988). An investigation of resistance in cabbage moth *(Plutella xylostella L.)* to pyrethroids in the Lockery Valley. Graduate Diploma Study Project. Agricultural College, Lawes, Queensland, Australia.

Anderson, P. and Lofqvist, J. (1996). Oviposition deterrents from potato, wheat germ, larval frass, and artificial diet for *Agrotis segetum* (Lepidoptera: Noctuidae). *Environmental Entomology* **25:** 653-658.

Andow, D. A. (1988). Management of weeds for insect manipulation in agroecosystems. *Weed management in agroecosystems: ecological approaches.* (ed. by M.A. Altieri and M.Z. Liebman), pp. 265-301. CRC, Boca Raton, Fla.

Andow, D. A. and Prokrym, D. R. (1990). Plant structural complexity and host-finding by a parasitoid. *Oecologia* **82:** 162-165.

Andow, D. A., Nicholson, A. G., Wien, H. C. and Wilsson, H. R. (1986). Insect populations on cabbage grown with living mulches. *Environmental Entomology* **15:** 293-299.

Ankersmit, G. W. (1953). DDT resistance in *Plutella maculipennis* (Curt.) (Lepidoptera) in Java. *Bulletin of Entomological Research* **44:** 421-425.

Arthur, A. P. (1962). Inflence of host tree on abundance of *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae), a polyphagus parasite of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.) (Lepidoptera: Olethreutidae). *The Canadian Entomologist* **94:** 337-347.

Askew, R. R. (1971). *Parasitic insects.* American Elsevier, New York.

Askew, R. R. and Shaw, M. R. (1986). Parasitoid communities: their size, structure and development. *Insect parasitoids* (ed. by J.k. Waage and D. Greathead.), pp. 225-264. Academic Press, London.

Atwal, A. S. (1955). Influence of temperature, photoperiod, and food on the speed of development, longevity, fecundity, and other qualities of the diamond-back moth, *Plutella maculipennis* (Curtis) (Lepidoptera: Tineidae). *Australian Journal of Zoology* **3:** 185-221.

Avilla, J. and Albajes, R. (1984). The influence of female age and host size on the sex ratio of the parasitoid *Opius concolor. Entomologia Experimentalis et Applicata* **35:** 43-47.

Bach, C. E. (1981). Host plant growth form and diversity: effects on abundance and feeding preference of a specialist herbivore, *Acalymma vittata* (Coleoptera: Chrysomelidae). *Oecologia* **50:** 370-375.

Bach, C. E. and Tabashnik, B. E. (1990). Effects of nonhost plant neighbors on population densities and parasitism rates of the diamondback moth (Lepidoptera: Plutellidae). *Environmental Entomology* **19:** 987-994..

Baker, G. J. (1994). Insecticidal resistance in *Plutella xylostella* (Lepidoptera: Plutellidae) populations in South Australia. *Managing insecticide resistance in diamondback moth : National workshop Proceedings* (ed. by P.M. Ridland and N.M. Endersby), pp. 11-13.

Cooperative Research Centre for Tropical Pest Management, Melbourne, Australia. Melbourne, Australia.

Baker, P. B., Shelton, A. M. and Andaloro, J. T. (1982). Monitoring of diamondback moth (Lepidoptera: Yponomeutidae) in cabbage with pheromones. *Journal of Economic Entomology* **75:** 1025-1028.

Barbosa, P. (1988). Some thoughts on "The evolution of host range". *Ecology* **69:** 912-915.

Bartlett, B. R. (1964). Patterns in the host-feeding habit of adult parasitic Hymenoptera. *Annals of the Entomological Society of America* **57:** 344-350.

Bastian, R. A. and Hart, E. R. (1990). First-generation parasitism of the mimosa webworm (Lepidoptera: Plutellidae) by *Elasmus albizziae* (Hymenoptera: Eulophidae) in an urban forest. *Environmental Entomology* **19:** 409-414.

Batra, S. W. T. (1982). Biological control in agroecosystems. *Science* **215:** 134-139.

Beck, N. G. and Cameron, P. J. (1990). Comparison of lepidopteran pest populations and their parasitoids in three vegetable brassicas. *Proceedings of the 43rd New Zealand Weed and Pest Control Conference*. pp. 21-25. Palmerston North, New Zealand, NZ Weed and Pest Control Society.

Beirne, B. P. (1971). Pest insects of annual crop plants in Canada. 1. Lepidoptera. *Plutella maculipennis* (Curt), the diamondback moth. *Memoirs of the Entomological Society of Canada* **78:** 38-39.

Bellows, T. S., Jr. and Birley, M. H. (1981). Estimating developmental and mortality rates and stage recruitment from insect stage frequency data. *Researches on Population Ecology* **23:** 232-244.

Bellows, T. S., Jr., Ortiz, M., Owens, J. C. and Huddleston, E. W. (1982). A model for analyzing insect stage frequency data when mortality varies with time. *Researches on Population Ecology* **24:** 142-156.

Bellows, T. S., Jr., van Driesche, R. G. and Elkinton, J. S. (1989). Extensions to Southwood and Jepson's graphical method of estimating numbers entering a stage for calculating mortality due to parasitism. *Researches on Population Ecology* **31:** 169-184.

Bellows, T. S., Jr., van Driesche, R. G. and Elkinton, J. S. (1992). Life-table construction and analysis in the evaluation of natural enemies. *Annual Review of Entomology* **37:** 587-614.

Bernays, E. A. and Chapman, R. F. (1994). *Host-plant selection by phytophagous insects.* Chapman and Hall, New York.

Bhalla, O. P. and Dubey, J. K. (1986). Bionomics of the diamondback moth in the northwestern Himalaya. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 55-61. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Birley, M. (1977). The estimation of insect density and instar survivorship functions from census data. *Journal of Animal Ecology* **46:** 497-510.

Boling, J. C. and Pitre, H. N. (1971). Hostal plant preference for oviposition by *Trichoplusia ni* (Lepidoptera: Noctuidae) and efficiency of *Apanteles marginiventris* as a populational regulator of *T. ni* in field-cage tests. *Journal of Economic Entomology* **64:** 411-412.

Bonnemaison, L. (1965). Insects pests of crucifers and their control. *Annual Review of Entomology* **10:** 232-256.

Boucek, Z. (1988). *Australian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species.* C.A.B, International., Wallingford.

Butt, R. A. and McEwen, F. L. (1981). Seasonal populations of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), in relation to day-degree accumulation. *The Canadian Entomologist* **113:** 127-131.

Caprio, M. A. and Tabashnik, B. E. (1992). Allozymes used to estimate gene flow among populations of diamondback moth (Lepidoptera: Plutellidae) in Hawaii. *Environmental Entomology* **21:** 808-816.

Carde, R. T., Comeau, A., Baker, T. C. and Roelofs, W. L. (1975). Moth mating periodicity: temperature regulates the circadian gate. *Experientia* **31:** 46-48.

Cates, R. G., Henderson, C. B. and Redak, R. A. (1987). Responses of the western spruce budworn to varying levels of nitrogen and terpenes. *Oecologia* **73:** 312-316.

Charnov, E. L. and Skinner, S. W. (1985). Complementary approaches to the understanding of parasitoid oviposition decisions. *Environmental Entomology* **14:** 383-391.

Chelliah, S. and Srinivasan, K. (1986). Bioecology and management of diamondback moth in India. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 63-76. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Cheng, E. Y. (1986). The resistance, cross resistance, and chemical control of diamondback moth in Taiwan. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 329-345. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Cheng, E. Y. (1988). Problems of control of insecticide-resistant *Plutella xylostella*. *Pesticide Science* **23:** 177-188.

Chew, F. S. (1975). Coevolution of pierid butterflies and their cruciferous foodplants. I. The relative quality of available resources. *Oecologia* **20:** 117-128.

Chisholm, M. D., Underhill, E. W. and Steck, W. F. (1979). Field trapping of the diamondback moth *Plutella xylostella* using synthetic sex attractants. *Environmental Entomology* **8:** 516-518.

Choi, I.-H., Yoo, J.-K. and Na, S.-Y. (1992). Studies on the ecological characteristics and effect of chemical control to diamondback moth, *Plutella xylostella* L. *Research Reports of the Rural Development Administration, Crop Protection* **34:** 40-47.

Chu, Y. I. (1986). The migration of diamondback moth. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 77-81. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Chua, T. H. and Ooi, P. A. C. (1986). Evaluation of three parasites in the biological control of diamondback moth in the Cameron Highlands, Malaysia. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 173-184. Asian Vegetable Research and Development Centre, Shanhua, Tawain.

Cordero, J. and Cave, R. D. (1992). Natural enemies of *Plutella xylostella* (Lep: Plutellidae) on crucifers in Honduras. *Entomophaga* **37:** 397-407.

Courtney, S. P. and Duggan, A. E. (1983). The population biology of the orange tip butterfly *Anthocharis cardamines* in Britain. *Ecological Entomology* **8:** 271-281.

Courtney, S. P. and Kibota, T. T. (1990). Mother doesn't know best: selection of hosts by ovipositing insects. *Insect/plant interactions* (ed. by E.A. Bernays), pp. CRC Press, Boca Raton, Florida.

Craig, T. P., Itami, J. K. and Price, P. W. (1989). A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology* **70:** 1691-1699.

Cromartie, W. J. (1975). The effect of stand size and vegetational background on the colonization of cruciferous plants by herbivorous insects. *Journal of Applied Ecology* **12:** 517-533.

Daigle, C. J., Boethel, D. J. and Fuxa, J. R. (1990). Parasitoids and pathogens of soybean looper and velvetbean caterpillar (Lepidoptera: Noctuidae) in soybeans in Louisiana. *Environmental Entomology* **19:** 746-752.

Daly, J. C. and Fitt, G. P. (1993). The need to validate monitoring techniques for insect populations. *Pest control & sustainable agriculture* (ed. by S.A. Corey, D.J. Dall and W.M. Milne), pp. 414-416. CSIRO, Canberra.

David, W. A. L. and Gardiner, B. O. C. (1966). Mustard oil glucosides as feeding stimulants for *Pieris brassica* larvae in a semi-synthetic diet. *Entomologia Experimentalis et Applicata* **9:** 247-255.

Day, W. H. (1994). Estimating mortality caused by parasites and diseases of insects: comparisons of the dissection and rearing methods. *Environmental Entomology* **23:** 543-550.

de Jong, P. W. and van Alphen, J. J. M. (1989). Host size selection and sex allocation in *Leptomastix dactylopii*, a parasitoid of *Planococcus citri*. *Entomologia Experimentalis et Applicata,* **50:** 161-169.

DeBach, P. and Rosen, D. (1991). *Biological Control by Natural Enemies.* Cambridge University Press, Cambridge.

Dempster, J. P. (1956). The estimation of the numbers of individuals entering each stage during the development of one generation of an insect population. *Journal of Animal Ecology* **25:** 1-5.

Dicke, M., van Lenteren, J. C., Boskamp, G. F. J. and van Dongen-van Leeuwen, E. (1984). Chemical stimuli in host-habitat location by *Leptopilina heterotoma* (Thompson) (Hymenoptera: Eucoilidae) a parasite of *Drosophila*. *Journal of Chemical Ecology* **10:** 695-712.

Endersby, N. M. and Morgan, W. C. (1991). Alternatives to synthetic chemical insecticides for use in crucifer crops. *Biological Agriculture and Horticulture* **8:** 33-52.

Esmaili, M. and Wilde, G. (1971). Behaviour of the parasite *Aphelinus asychii* in relation to the greenbug and certain hosts. *Environmental Entomology* **1:** 266-268.

Fitton, M. and Walker, A. (1992). Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop* (ed. by N.S. Talekar), pp. 225-232. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Flanders, S. E. (1942). Abortive development in parasitic Hymenoptera, induced by the food-plant of the insect host. *Journal of Economic Entomology* **35:** 834-835.

Ford, E. D. (1975). Competition and stand structure in some even-aged plant monocultures. *Journal of Ecology* **63:** 311-333.

Fox, L. R. and Eisenbach, J. (1992). Contrary choices: possible exploitation of enemy-free space by herbivorous insects in cultivated vs. wild crucifers. *Oecologia* **89:** 574-579.

Fox, L. R. and Morrow, P. A. (1981). Specialization: species property or local phenomenon? *Science* **211:** 517-533.

Fox, L. R., Letourneau, D. K., Eisenbach, J. and van Nouhuys, S. (1990). Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. *Oecologia* **83:** 414-419.

French, R. A. and White, J. H. (1960). The diamondback moth outbreak of 1958. *Plant Pathology* **9:** 77-84.

Gauld, I. D. (1984). *An Introduction to the Ichneumonidae of Australia.* British Museum (Natural History), London.

Goodwin, S. (1979). Changes in numbers in the parasitoid complex associated with the diamond-back moth, *Plutella xylostella* (L.) (Lepidoptera), in Victoria. *Australian journal of Zoology* **27:** 981-989.

Gould, F. and Stinner, R. E. (1984). Insects in heterogeneous habitats. *Ecological Entomology* (ed. by C.B. Huffaker and R.L. Rabb), pp. 427-449. Wiley, New York.

Gould, J. R. (1990). *Estimating the impact of parasitoids on the dynamics of populations of gypsy moths.* PhD Thesis. University of Massachusetts, Amherst.

Gould, J. R., Elkinton, J. S. and Wallner, W. E. (1990). Density-dependent suppression of experimentally created gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), populations by natural enemies. *Journal of Animal Ecology* **59:** 213-233.

Grant, A. J., Borroni, P. F. and O'connell, R. J. (1996). Different seasonal rearing conditions do not affect pheromone-sensitive receptor neurons of the adult cabbage looper moth, *Trichplusia ni*. *Physiological Entomology* **21:** 59-63.

Greathead, D. J. (1986). Parasitoids in classical biological control. *Insect parasitoids, 13th Symposium of the Royal Entomological Society* (ed. by J.K. Waage and D.J. Greathead), pp. 289-318. Inc., London.

Gupta, P. D. and Thorsteinson, A. J. (1960a). Food plant relationships of the diamond-back moth (*Plutella maculipennis* (Curt.)). I. Gustation and olfaction in relation to botanical specificity of larva. *Entomologia Experimentalis et Applicata* **3:** 241-250.

Gupta, P. D. and Thorsteinson, A. J. (1960b). Food plant relationships of the diamond-back moth (*Plutella maculipennis* (Curt.)). II. Sensory regulation of oviposition of the adult female. *Entomologia Experimentalis et Applicata* **3:** 305-314.

Gupta, V. K. (1964). Taxonomic identity of two ichneumonid parasites of *Plutella maculipennis* (Curt.) in Australia. *Mushi* **38:** 37-38.

Hall, R. W. and Ehler, L. E. (1979). Rate of establishment of natural enemies in classical biological control. *Bulletin of the Entomological Society of America* **25:** 280-282.

Hallett, R. H., Zilahi-Balogh, R., Angerilli, N. P. D. and Borden, J. H. (1993). Development of a pest management system for diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) in a third-world country-considerations for sustainability. *Pest control & sustainable agriculture* (ed. by S.A. Corey, D.J. Dall and W.M. Milne), pp. 85-88. CSIRO, Canberra.

Harcourt, D. G. (1954). *The biology and ecology of the diamondback moth, Plutella maculipennis (Curt.), in eastern Ontario.* PhD Thesis. Cornell University, Ithaca.

Harcourt, D. G. (1957). Biology of diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae) in Eastern Ontario. II. Life history, behaviour, and host relationship. *The Canadian Entomologist* **89:** 554-564.

Harcourt, D. G. (1963). Major mortality factors in the population dynamics of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae). *Memoirs of the Entomological Society of Canada* **32:** 55-56.

Harcourt, D. G. (1986). Population dynamics of the diamondback moth in Southern Ontario. *Diamondback moth management : Proceedings of the First International*

Workshop (ed. by N.S. Talekar and T.D. Grigg), pp. 3-15. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Harcourt, D. G. and Cass, L. M. (1966). Photoperiodism and fecundity in *Plutella maculipennis* (Curt.). *Nature* **210:** 217-218.

Hardy, J. E. (1938). *Plutella maculipennis* Curt., its natural and biological control in England. *Bulletin of Entomological Research* **29:** 343-372.

Harper, J. L. and McNaughton, I. H. (1962). The comparative biology of closely related species living in the same area. VII. Interference between individuals in pure and mixed populations of *Papaver* species. *The New Phytologist* **61:** 175-188.

Hassell, M. P. and Godfray, H. C. j. (1992). The population biology of insect parasitoids. *Natural Enemies, the population biology of predators and diseases* (ed. by M.J. Crawley), pp. 265-292. Blackwell Scientific Publications, Oxford, London.

Haukioja, E. (1993). Effects of food and predation on population dynamics. *Caterpillars, ecological and evolutionary constraints on foraging* (ed. by E.S. Stamp and T.M. Casey), pp. 425-447. Chapman and Hall, New York.

Herminanto, H. (1995). *Potential of parasitoids for the control of cabbage moth in augmentative releases.* M.Ag.Sc. Thesis. The University of Adelaide, Adelaide.

Hillyer, R. J. and Thorsteinson, A. J. (1971). Influence of the host plant or males on programming of oviposition in the diamondback moth (*Plutella maculipennis* (Curt.): Lepidoptera). *Canadian Journal of Zoology* **49:** 983-990.

Hirashima, Y., Abe, M. and Tadauchi, O. (1989). The hymenopterous parasitoids of the diamondback moth, *Plutella xylostella* (Lepidoptera, Yponomeutidae) in Japan. *Esakia* **28:** 63-73.

Holm, L., Pancho, J. V., Herberger, J. P. and Plucknett, D. L. (1979). *A geographical atlas of world weeds.* Wiley and Sons Publishing, New York.

Honda, K. (1992). Hibernation and migration of the diamondback in north Japan. *Managemnet of Diamondback moth and other crucifer pests : Proceedings of the Second*

International workshop (ed. by N.S. Talekar), pp. 43-50. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Honda, K., Miyahara, Y. and Kegasawa, K. (1992). Seasonal abundance and the possibility of spring immigration of the diamondback moth, *Plutella xylostella* (LINNAEUS) (Lepidoptera: Yponomeutidae), in Morioka City, Northern Japan. *Applied Entomology and Zoology* **27:** 517-525.

Hopper, K. R. (1986). Preference, acceptance and fitness components of *Microplitis croceipes* (Hymenoptera: Braconidae) attacking various instars of *Heliothis virescens* (Lepidoptera: Noctuidae). *Environmental Entomology* **15:** 274-280.

Hopper, K. R. and Roush, R. T. (1993). Mate finding, dispersal, number released, and the success of biological control introductions. *Ecological Entomology* **18:** 321-331.

Horn, D. J. (1981). Effect of weedy backgrounds on colonization of collards by green peach aphid, *Myzus persicae*, and its major predators. *Environmental Entomology* **10:** 285-289.

Horn, D. J. (1987). Vegetational background and parasitism of larval diamond-back moth on collards. *Entomologia Experimentalis et Applicata* **43:** 300-303.

Horner, J. D. and Abrahamson, W. G. (1992). Influence of plant genotype and environment on oviposition preference and offspring survival in a gallmaking herbivore. *Oecologia* **90:** 323-332.

Huffaker, C. B. and Messenger, P. S. (1976). *Theory and practice of biological control.* Academic Press, New York.

Huffaker, C. B. and Rabb, R. L. (1984). *Ecological Entomology.* John Wiley and Sons, New York.

Iga, M. (1985). The seasonal prevalence of occurrence and the life tables of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae). *Japanese Journal of Applied Entomology and Zoology* **29:** 119-125. (In Japanese with English summary).

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics* **21:** 56-89.

Jansson, R. K. and Smilowitz, Z. (1986). Influence of potato persistence, foliar biomass and foliar nitrogen on abundance of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Environmental Entomology* **15:** 726-732.

Jessop, J. P. and Toelken, H. R. (1986). *Flora of South Australia. Part I. Lycopodiaceae-Rosaceae.* South Australian Government Printing Division, Adelaide.

Juliano, S. A. (1982). Influence of host age on host acceptability and suitability for a species of *Trichogramma* (Hymenoptera: Trichgrammatidae) attacking aquatic Diptrea. *The Canadian Entomologist* **114:** 713-720.

Kareiva, P. (1983). Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement. *Variable plants and herbivores in natural and managed systems* (ed. by R.F. Denno and M.S. McClure), pp. 259-289. Academic, New York.

Kauffman, W. C. and Kennedy, G. G. (1989). Relationship between trichome density in tomato and parasitism of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs by *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). *Environmental Entomology* **18:** 698-704.

Kay, Q. O. N. (1976). Preferential pollination of yellow-flowered morphs of *Raphanus raphnistrum* by *Pieris* and *Eristalis* spp. *Nature* **26:** 230-232.

Kiritani, K. and Nakasuji, F. (1967). Estimation of the stage-specific survival rate in the insect population with overlapping stages. *Researches on population Ecology* **9:** 143-152.

Knipling, E. F. (1992). *Principles of insect parasitism analyzed from new perspectives: Practical implications for regulating insect populations by biological means.* United States Department of Agriculture, Agricultural Handbook.

Koshihara, T. (1986). Diamondback moth and its control in Japan. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D.Griggs), pp. 43-53. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Kouamè, K. L. and Mackauer, M. (1991). Influence of aphid size, age and behaviour on host choice by the parastoid wasp *Ephedrus californicus*: A test of host-size models. *Oecologia* **88:** 197-203.

Kozar, F., Brown, M. W. and Lightner, G. (1994). Spatial distribution of homopteran pests and beneficial insects in an orchard and its connection with ecological plant protection. *Journal of Applied Entomology* **117:** 519-529.

Lardner, R. M. and Hales, D. (1990). Histopathology of an aphid, *Schoutedenia lutea*, parasitized by a gall midge, *Pseudendaphis* sp. *Entomophaga* **35:** 557-567.

Leather, S. R. (1994). Life history traits of insect herbivores in relation to host quality. *Insect-Plant Interactions* (ed. by E.A. Bernays), pp. 176-207. CRC Press, Inc., London.

Legaspi, B. A. C. J. (1986). Host discrimination in two species of ichneumonid wasps, *Diadegma* spp., attacking larvae of *Plutella xylostella*. *Entomologia Experimentalis et Applicata* **41:** 79-82.

Letourneau, D. K. and Fox, L. R. (1989). Effects of experimental design and nitrogen on cabbage butterfly oviposition. *Oecologia* **80:** 211-214.

Lewis, A. C. (1984). Plant quality and grasshopper feeding: effects of sunflower condition on preference and performance in *Melanoplus differentalis*. *Ecology* **65:** 836-843.

Lewis, W. J. and Vinson, S. B. (1971). Suitability of certain *Heliothis* (Lepidoptera: Noctuidae) as hosts for the parasite *Cardiochiles nigriceps*. *Annals of the Entomological Society of America* **64:** 970-972.

Lim, G. S. (1986). Biological control of diamondback moth. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 159-171. Asian Vegetable Research and Development Centre, Shanhua, Tawain.

Lin, J., Dickson, M. H. and Eckenrode, C. J. (1984). Resistance of *Brassica* lines to the diamondback moth (Lepidoptera: Yponomeutidae) in the field, and inheritance of resistance. *Journal of Economic Entomology* **77:** 1293-1296.

Liu, M. Y. and Sun, C. N. (1984). Rearing diamondback moth (Lepidoptera: Yponomeutidae) on rape seedlings by a modification of the Koshihara and Yamada method. *Journal of Economic Entomology* **77:** 1608-1609.

Liu, M. Y., Tzeng, Y. J. and Sun, C. N. (1982). Insecticide resistant in the diamondback moth. *Journal of Economic Entomology* **75:** 153-155.

Liu, S.-S., Morton, R. and Hughes, R. D. (1984). Oviposition preferences of a hymenopterous parasite for certain instars of its aphid host. *Entomologia Experimentalis et Applicata* **35:** 249-254.

Lloyd, D. C. (1940). Host selection by Hymenopterous parasites of the moth *Plutella maculipennis* Curtis. *Proceedings of the Royal Society of London* **128:** 451-484.

Lopez, E. R. and van Driesche, R. G. (1989). Direct measurement of host and parasitoid recruitment for assessment of total losses due to parasitism in a continuously breeding species, the cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). *Bulletin of Entomological Research* **79:** 47-59.

Lopez, E. R., Ferro, D. N. and van Driesche, R. G. (1993). Direct measurement of host and parasitoid recruitment for assessment of total losses due to parasitism in the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and *Miopharus doryphorae* (Riley) (Diptera: Tachinidae). *Biological Control* **3:** 85-92.

Luck, R. F., Merle Shepard, B. and Kemmore, P. E. (1988). Experimental methods for evaluating arthropod natural enemies. *Annual Review of Entomology* **33:** 367-391.

Luther, G. C., Valenzuela, H. R. and Defrank, J. (1996). Impact of cruciferous trap crops on Lepidopteran pests of cabbage in Hawaii. *Environmental Entomology* **1:** 39-47.

Maa, C. J. W. (1986). Ecological approach to male diamondback moth response to sex pheromone. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 109-123. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

MacGarvin, M. (1982). Species-area relationships of insects on host plants: herbivores on rosebay willowherb. *Journal of Animal Ecology* **51:** 207-223.

Mackenzie, J. M. B. (1958). Invasion of diamondback moth (*Plutella maculipennis* Curtis). *Journal of Entomological Science* **91:** 247-250.

Manly, B. F. J. (1974). Estimation of stage-specific survival rates and other parameters for insect populations developing through several life stages. *Oecologia* **15:** 277-285.

Manly, B. F. J. (1976). Extensions to kiritani and Nakasuji's method for analyzing insect stage-frequency data. *Researches on Populations Ecology* **17:** 191-199.

Manly, B. F. J. (1977). A further note on Kiritani and Nakasuji's model for stage frequency data including comments on the use of Tukey's jacknife technique for estimating variances. *Researches on Population Ecology* **18:** 177-186.

Manly, B. F. J. (1987). A multiple regression method for analyzing stage-frequency data. *Researches on Population Ecology* **29:** 119-127.

Manly, B. F. J. (1989). A review of methods for the analysis of stage-frequency data. *Estimation and analysis of insect populations* (ed. by L.L. McDonald, L.B.J. Manly, J. Lockwood and J. Logan), pp. 3-69. Springer-Verlag, New York.

Mappes, J. and Makela, I. (1993). Egg and larval load assessment and its influence on oviposition behaviour of the leaf beetle *Galerucella nymphaeae. Oecologia* **93:** 38-41.

Marrone, P. G. (1982). An inexpensive technique for controlling soil moisture in laboratory experiments with insects requiring growing plants. *Pedobiologia* **24:** 121-127.

Marsh, O. H. (1917). The life history of *Plutella maculipennis*, the diamondback moth. *Journal of Agricultural Research* **10:** 1-10.

Mattson, W. J. J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11:** 119-161.

May, M. L. and Ahmad, S. (1983). Host location in the Colorado potato beetle: searching mechanisms in relation to oligophagy. *Herbivorous insects: host-seeking behaviour and mechanisms* (ed. by S. Ahmad), pp. 173-199. Academic Press, New York.

Mclaughlin, J. R., Mitchell, E. R. and Kirsch, P. (1994). Mating disruption of diamondback moth (Lepidoptera: Plutellidae) in cabbage: reduction of mating and suppression of larval populations. *Journal of Economic Entomology* **87:** 1198-1204.

Meyrick, E. (1928). *A revised handbook of British Lepidoptera.* Watkins and Doncaster, London.

Minkenberg, O. P. J. M. and Ottenheim, J. J. G. W. (1990). Effect of leaf nitrogen content of tomato plants on preference and performance of a leafmining fly. *Oecologia* **83:** 291-298.

Miyata, T., Saito, T. and Noppun, V. (1986). Studies on the mechanism of diamondback moth resistance to insecticides. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 347-357. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Moller, J. (1988). Investigations on a laboratory culture of the diamond-back moth, *Plutella maculipennis* (Curt.) (Lep., Tineidae). I. Life history parameters: Hatching, pupation, emergence, copulation and oviposition. *Journal of Applied Entomology* **105:** 360-373.

Monteith, L. G. (1955). Host preferences of *Drino bobemica* Mens. (Diptera: Tachinidae) with particular refernce to olfactory responses. *The Canadian Entomologist* **87:** 509-530.

Muhamad, O., Tsukuda, R., Oki, Y., Fujisaki, K. and Nakasuji, F. (1994). Influences of wild crucifers on life history traits and flight ability of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Researches on Population Ecology* **36:** 53-62.

Mustata, G. (1992). Role of parasitoid complex in limiting the population of diamondback moth in Moldavia, Romania. *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop* (ed. by N.S. Talekar), pp. 203-211. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Myers, J. H. (1985). Effect of physiological condition of host plant on the ovipositional choice of the cabbage butterfly, *pieris rapae*. *Journal of Animal Ecology* **54:** 193-204.

Nettles, W. C. J. (1980). Adult *Eucelatoria* sp.; response to volatiles from cotton and okra plants and from larvae of *Heliothis virescens*, *Spodoptera eridania* and *Estigmene acrea*. *Environmental Entomology* **9:** 759-763.

Nielsen, J. K. (1978). Host plant discrimination within Cruciferae: Feeding responses of four beetles (Coleoptera: Chrysomelidae) to glucosinolates, cucurbitacins and cardenolides. *Entomologia Experimentalis et Applicata* **24:** 41-54.

Nielsen, J. K. (1989). The effect of glucosinolates on responses of young *Phyllotreta nemorum* larvae to non-host plants. *Entomologia Experimentalis et Applicata* **51:** 249-259.

Nordlund, D. A. and Sauls, C. E. (1981). Kairomones and their use for management of entomophagus insects. XI. Effect of host plants on kairomonal activity of frass from *Heliothis zea* larvae for the parasitoid *Microplitis croceipes*. *Journal of Chemical Ecology* **7:** 1057-1061.

Nordlund, D. A., Jones, R. L. and Lewis, W. J. (1981). *Semiochemicals, their role in pest control.* Wiley, New York.

Oatman, E. R. and Platner, G. R. (1969). An ecological study of insect populations on cabbage in southern California. *Hilgardia* **40:** 1-40.

Ohno, T., Asayama, T. and Ichikawa, K. (1992). Evaluation of communication disruption method for suppressing diamondback moth infestations at two different areas using synthetic sex pheromone. *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop* (ed. by N.S. Talekar), pp. 109-114. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Okuda, M. S. and Yeargan, K. V. (1988). Habitat partitioning by *Telenomus podisi* and *Trissolcus euschisti* (Hymenoptera: Scelionidae) between herbaceous and woody host plants. *Environmental Entomology* **17:** 795-798.

Olsson, K. and Jonasson, T. (1994). Leaf feeding by caterpillars on white cabbage cultivars with different 2-propenyl glucosinolate (sinigrin) content. *Journal of Applied Entomology* **118:** 197-202.

Ooi, P. A. C. (1986). Diamondback moth in Malaysia. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 25-34. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Ooi, P. A. C. (1992). Trends in biological control in Malaysia. *Pest management and the environment in 2000* (ed. by A. Aziz, S.A. Kadir and H.S. Barlow), pp. 341-347. C. A. B. International, U.K.

Opp, S. B. and Luck, R. F. (1986). Effects of host size on selected fitness components of *Aphytis melinus* and *A. lingnanensis* (Hymenoptera: Aphelinidae). *Annals of the Entomological Society of America* **79:** 700-704.

Palaniswamy, P., Gillot, C. and Slater, G. P. (1986). Attraction of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) by volatile compounds of canola (*Brassica campestris* cultivar Tobin), white mustard (*Brassica hirta* cultivar Ochre), and faba bean (*Vicia faba*). *The Canadian Entomologist* **118:** 1279-1286.

Panda, N. and Khush, G. (1995). *Host plant resistance to insects.* CAB International, Wallingford.

Perfect, T. J. (1992). IPM in 2000. *Pest management and the environment in 2000* (ed. by A. Aziz, S.A. Kadir and H.S. Barlow), pp. 47-53. C. A. B. International, U.K.

Petersen, J. J. (1986). Evaluating the impact of pteromalid parasites on filth fly populations associated with confined livestock installations. *Entomological Society of America Misc. Publ.* **61:** 52-56.

Petersen, J. J. and Watson, D. W. (1992). Comparison of sentinel and naturally occurring fly pupae to measure field parasitism by pteromalid parasitoids (Hymenoptera). *Biological control* **2:** 244-248.

Pimentel, D. (1961a). Species diversity and insect population outbreaks. *Annals of the Entomological Society of America* **54:** 76-86.

Pimentel, D. (1961b). The influence of plant spatial patterns on insect populations. *Annals of the Entomological Society of America* **54:** 61-69.

Pimentel, D. (1961c). An evaluation of insect resistance in broccoli, brussels sprouts, cabbage, collards and kale. *Journal of Economic Entomology* **54:** 156-158.

Pittendrigh, B. R. and Pivnick, K. A. (1993). Effects of a host plant, *Brassica juncea*, on calling behaviour and egg maturation in the diamondback moth, *Plutella xylostella*. *Entomologia Experimentalis et Applicata* **68:** 117-126.

Pivnick, K. A., Jarvis, B. J., Gillott, C., Slater, G. P. and Underhill, E. W. (1990a). Daily patterns of reproductive activity and the influence of adult density and exposure to host plants on reproduction in the diamondback moth (Lepidoptera: plutellidae). *Environmental Entomology* **19:** 587-593.

Pivnick, K. A., Jarvis, B. J., Slater, G. P., Gillott, C. and Underhill, E. W. (1990b). Attraction of the diamondback moth (Lepidoptera: Plutellidae) to volatiles of oriental mustard: the influence of age, sex, and prior exposure to mates and host plants. *Environmental Entomology* **19:** 704-709.

Poelking, A. (1992). Diamondback moth in the Philippines and its control with *Diadegma semiclausum*. *Diamondback moth and other crucifer pests : Proceeding of the Second International workshop* (ed. by N.S. Talekar), pp. 271-278. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Prescott, A. (1988). *It's Blue with five petals, wildflowers of the Adelaide region*. Ann Prescott Publisher, South Australia.

Preszler, R. W. and Price, P. W. (1988). Host quality and sawfly population: a new approach to life table analysis. *Ecology* **69:** 2012-2020.

Price, P. W. (1972). Parasitoids utilizing the same host: adaptive nature of differences in size and form. *Ecology* **53:** 190-195.

Putnam, L. G. (1968). Experiments in the quantitative relations *Diadegma insularis* (Hymenoptera: Ichneumonidae) and *Microplitis plutellae* (Hymenoptera: Braconidae) with their host *Plutella maculipennis* (Lepidoptera: Plutellidae). *The Canadian Entomologist* **100:** 12-16.

Putnam, L. G. (1973). Effects of the larval parasites *Diadegma insularis* and *Microplitis plutellae* on the abundance of the diamondback moth in Saskatchewan rape and mustard crops. *Canadian Journal of Plant Science* **53:** 911-914.

Ralph, C. P. (1977). Effect of host plant density on populations of a specialized, seed-sucking bug, *Oncopeltus fasciatus*. *Ecology* **58:** 799-809.

Rausher, M. D. (1983a). Alteration of oviposition behavior by *Battus philenor* butterflies in response to variation in host-plant density. *Ecology* **64:** 1028-1034.

Rausher, M. D. (1983b). Conditioning and genetic variation as causes of individual variation in the oviposition behaviour of the tortoise beetle, *Deloyala guttata*. *Animal behaviour* **31:** 743-747.

Reed, D. W., Pivnick, K. A. and Underhill, E. W. (1989). Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella*, present in three species of Brassicaceae. *Entomologia Experimentalis et Applicata* **53:** 277-286.

Reeve, J. D. (1987). Foraging behaviour of *Aphytis melinus* : effects of patch density and host size. *Ecology* **68:** 530-538.

Renwick, J. A. A. and Raadke, C. D. (1990). Plant constituents mediating oviposition by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Phytophaga* **3:** 37-46.

Richards, O. W., Waloff, N. and Spradbery, J. P. (1960). The measurement of mortality in an insect population in which recruitment and mortality widely overlap. *Oikos* **11:** 306-310.

Riethmacher, G. W. and Kranz, J. (1994). Development of disease incidence of Entomophthoraceae in field populations of *Plutella xylostella* in the Philippines. *Journal of Plant Diseases and Protection* **101:** 357-367.

Risch, S. J. (1979). A comparison, by sweep sampling, of the insect fauna from corn and sweet potato monocultures and dicultures in Costa Rica. *Oecologia* **42:** 195-211.

Risch, S. J., Andow, D. and Altieri, M. A. (1983). Agroecosystem diversity and pest control: data, tentative conclusions, and new research directions. *Environmental Entomology* **12:** 625-629.

Roff, D. A. and Fairbairn, D. J. (1991). Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. *American Zoologist* **31:** 243-251.

Root, R. B. (1973). Organization of a plant-arthropod association in simple and diverse habitats. The fauna of collards (*Brassica oleracea*). *Ecological Monographs* **43:** 45-124.

Rosenheim, J. A. and Rosen, D. (1991). Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis*: distinguishing the influences of egg load and experience. *Journal of Animal Ecology* **60:** 873-893.

Rosenthal, G. A. and Janzen, D. H. (1979). *Herbivores: their interaction with secondary plant metabolites.* Academic Press, New York.

Ruesink, W. G. (1975). Estimating time-varying survival of arthropod life stages from population density. *Ecology* **56:** 244-247.

Ryan, R. B. and Medley, R. D. (1970). Test release of *Itoplectis quadricingulatus* against European pine shoot moth in an isolated infestation. *Journal of Economic Entomology* **63:** 1390-1392.

Salinas, P. J. (1986). Studies on diamondback moth in Venezuela with reference to other Latinamerican countries. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 17-24. Asian Vegetable Research and Development centre, Shanhua, Taiwan.

Sands, D. P. A. (1993). Effects of confinement on parasitoid/host interactions: interpretation and assessment for biological control of arthropod pests. *Pest control & sustainable agriculture* (ed. by S.A. Corey, D.J. Dall and W.M. Milne), pp. 196-199. CSIRO, Canberra.

Sarnthoy, O., Keinmeesuke, P., Sinchaisri, N. and Nakasuji, F. (1989). Development and reproductive rate of the diamondback moth, *Plutella xylostella* from Thailand. *Applied Entomology and Zoology* **24:** 202-208.

SAS (1993). *SAS User's Guide: Statistics, release 6.10.* Institute Inc., Cary, NC.

Sastrodihardjo, S. (1986). Diamondback moth in Indonesia. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 35-41. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Sastrosiswojo, S. and Sastrodihardjo, S. (1986). Status of biological control of diamondback moth by introduction of parasitoid *Diadegma eucerophaga* in Indinesia. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 185-194. Asian Vegetable Reseach and Development Centre, Shanhua, Taiwan.

Scriber, J. M. (1977). Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of *Hyalophora cecropia* (Lepidoptera: Saturnidae). *Oecologia* **28:** 269-287.

Scriber, J. M. (1984). Host-plant suitability. *Chemical ecology of insects* (ed. by W.J. Bell and R.T. Carde), pp. 159-202. Chapman and Hall, London.

Scriber, J. M. and Slansky, F., Jr. (1981). The nutritional ecology of immature insects. *Annual Review of Entomology* **26:** 183-211.

Sequeira, R. and Mackauer, M. (1994). Variation in selected life-history parameters of the parasitoid wasp, *Aphidius ervi*: influence of host developmental stage. *Entomologia Experimentalis et Applicata* **71:** 15-22.

Shelton, A. M., Hoy, C. W., North, R. C., Dickson, M. H. and Barnard, J. (1988). Analysis of resistance in cabbage varieties to damage by Lepidoptera and Thysanoptera. *Journal of Economic Entomology* **81:** 634-640.

Shirai, Y. (1993). Factors influencing flight ability of male adults of the diamondback moth, *Plutella xylostella*, with special reference to temperature conditions during the larval stage. *Applied Entomology and Zoology* **28:** 291-301.

Shirai, Y. and Nakamura, A. (1994). Dispersal movement of male adults of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), on cruciferous

vegetable fields, studied using the mark-recapture method. *Applied Entomology and Zoology* **29:** 339-348.

Shirai, Y. and Nakamura, A. (1995). Relationship between the number of wild males captured by sex pheromone trap and the population density estimated from a mark-recapture study in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* **30:** 543-549.

Simmonds, F. J. (1948). Some difficulties in determining by means of field samples the true value of parasitisc control. *Bulltin of Entomological Research* **39:** 435-440.

Sivapragasam, A., Ito, Y. and Saito, T. (1988). Population fluctuations of the diamondback moth, *Plutella xylostella* (L.) on cabbages in *Bacillus thuringiensis* sprayed and non-sprayed plots and factors affecting within-generation survival of immatures. *Researches on Population Ecology* **30:** 329-342.

Slansky, F. J. (1986). Nutritional ecology of endoparasitic insect and their hosts: an overview. *Journal of Insect Physiology* **32:** 255-261.

Smith, H. S. (1942). A race of *Comperiella bifasciata* successfully parasitises California red scale. *Journal of Economic Entomology* **35:** 809-812.

South Australian Year Book. (1995). *Australian Bureau of Statistics.* South Australian Office, Adelaide.

Southwood, T. R. E. (1978). *Ecological Methods with particular reference to the study of insect populations.* John Wiley and Sons, New York.

Southwood, T. R. E. and Jepson, W. F. (1962). Studies on the populations of *Oscinella frit.* L. (Diptera: Chloropidae) in the oat crop. *Journal of Animal Ecology* **31:** 481-495.

Spooner-Hart, R. (1993). Arthropods in the antipodes: pests and biological control in protected crops in Australia. *Working group "IPM glasshouses"* (ed. by J.C. van Lenteren), pp. 153-155. IOBC/WPRS *Bulletin* 16., Pacific Grove, California, USA.

Srinivasan, K. and Krishna Moorthy, P. N. (1992). Development and adoption of integrated pest management for major pests of cabbage using Indian mustard as a trap crop. *Diamondback moth and other crucifer pests : Proceedings of the Second International*

workshop (ed. by N.S. Talekar), pp. 511-521. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Stanton, M. L. (1983). Spatial patterns in the plant community and their effect upon insect search. *Herbivorous insects: host- seeking behavior and mechanisms* (ed. by S. Ahmad), pp. 125-155. Academic Press, New York.

Statistix (1992). *Statistix User's Manual, Version 4.* Analytical Software, St. Paul MN.

Stiling, P. D. (1990). Calculating the establishment rates of parasitoids in biological control. *American Entomologist* **36:** 225-230.

Sun, C. N. (1992). Insecticide resistance in diamondback moth. *Diamondback moth and other crucifer pests : Proceedings of the Second International Workshop* (ed. by N.S. Talek), pp. 419-426. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Sun, C. N., Wu, T. K., Chen, J. S. and Lee, W. T. (1986). Insecticide resistance in diamondback moth. *Diamondback moth management : Proceedings of the First International Workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 359-371. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Syed, A. R. (1992). Management of pests and diseases of vegetable crops in Malaysia in 2000. *Pest management and the environment in 2000* (ed. by A. Aziz, S.A. Kadir and H.S. Barlow), pp. 213-230. C. A. B. International, U.K.

Syme, P. A. (1975). The effect of flowers on the longevity and fecundity of two native parasites of the european pine shoot moth in Ontario. *Environmental Entomology* **4:** 337-340.

Tabashnik, B. E. (1985). Deterrence of diamondback moth (Lepidoptera: Plutellidae) oviposition by plant compounds. *Environmental Entomology* **14:** 575-578.

Tabashnik, B. E. and Mau, R. F. L. (1986). Suppression of diamondback moth (Lepidoptera: Plutellidae) oviposition by overhead irrigation. *Journal of Economic Entomology* **79:** 189-191.

Tabashnik, B. E., Cushing, N. L. and Johnson, M. W. (1987). Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: intra-island variation and cross-resistance. *Journal of Economic Entomology* **80:** 1091-1099.

Tahvanainen, J. O. and Root, R. B. (1972). The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomellidae). *Oecologia* **10:** 321-346.

Talekar, N. S. and Griggs, T. D., eds. (1986). *Diamondback moth management : Proceedings of the First International Workshop.* Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Talekar, N. S. and Shelton, A. M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* **38:** 275-301.

Talekar, N. S. and Yang, J. C. (1991). Characteristics of parasitism of diamondbck moth by two larval parasites. *Entomophaga* **36:** 94-105.

Talekar, N. S. and Yang, J. C. (1993). Influence of crucifer cropping system on the parasitism of *Plutella xylostella* (Lep., Yponomeutidae) by *Cotesia plutellae* (Hym., Braconidae) and *Diadegma semiclausum* (Hym., Ichneumonidae). *Entomophaga* **38:** 541-550.

Talekar, N. S., ed. (1992). *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop.* Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Talekar, N. S., Lee, S. T. and Huang, S. W. (1986). Intercropping and modification of irrigation method for the control of diamondback moth. *Diamondback moth management : Proceedings of the First International workshop* (ed. by N.S. Talekar and T.D. Griggs), pp. 145-151. Asian Vegetable Research and Development Centre, Shanhua, Taiwan.

Talekar, N. S., Yang, H. C., Loess, s. T., Chen, B. S. and Sun, L. Y. (1985). *Annotated Bibliography of Diamonback Moth.* Asian Vegetable Research and Development Centre., Shanhua, Taiwan.

Tauber, M. J., Tauber, C. A. and Masaki, S. (1986). *Seasonal adaptations of insects.* Oxford University Press, New York.

Taylor, A. D. (1988). Host effects on functional and ovipositional responses of *Bracon hebetor*. *Journal of Animal Ecology* **57:** 173-184.

Thompson, J. (1978). Within-patch structure and dynamics in *Pastinaca sativa* and resource availability to a specialized herbivore. *Ecology* **59:** 443-448.

Thompson, W. R. (1946). A catalogue of parasites and predators of insect pests. *Imperial Parasite Service.* Canada. Section 1, part 8.

Thorsteinson, A. J. (1953). The chemotatic responses that determine host specificity in an oligophagous insect (*Plutella maculipennis* (Curt.) Lepidoptera). *Canadian Journal of Zoology* **31:** 52-57.

Torgensen, T. R. and Ryan, R. B. (1981). Field biology of *Telenomus californicus* Ashmead, an important egg parasitoid of Douglas fir tussock moth. *Annals of the Entomological Society of America* **74:** 185-186.

Uematsu, H. and Sakanoshita, A. (1989). Possible role of cabbage leaf wax bloom in suppressing diamondback moth *plutella xylostella* (Lepidoptera: Yponomeutidae) oviposition. *Applied Entomology and Zoology* **24:** 253-257.

Unal, I., Mohamed, M., Hong, L. W. and Rahman, S. A. (1993). Evaluation of synthetic sex pheromones for suppressing diamondback moth, *Plutella xylostella* (L). *Pest control & sustainable agriculture* (ed. by S.A. Corey, D.J. Dall and W.M. Milne), pp. 293-295. CSIRO, Canberra.

van Alphen, J. J. M. (1980). Aspects of the foraging behaviour of *Tetrastichus asparagi* Crawford and *Tetrastichus* spec. (Eulophidae), gregarious egg parasitoids of the asparagus beetles *Crioceris asparagi* L. and *C. duodecimpunctata* L. (Chrysomelidae). I. Host-species selection, host-stage selection and host discrimination. *Netherlands Journal of Zoology* **30:** 307-325.

van Alphen, J. J. M. and Vet, L. E. M. (1986). An evolutionary approach to host finding and selection. *Insect parasitoids* (ed. by J.K. Waage and D.J. Greathead), pp. 23-61. Academic Press, London.

van den Assem, J., Putters, F. A. and Prins, T. C. (1984). Host quality effects on sex ratio of the parasitic wasp *Anisopteromalus calandrae* (Chalcidoidea, Pteromalidae). *Netherlands Journal of Zoology* **34:** 33-62.

van den Berg, H., Nyambo, B. T. and Waage, J. k. (1990). Parasitism of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Tanzania: analysis of parasitoid-crop associations. *Environmental Entomology* **19:** 1141-1145.

van Driesche, R. G. (1983). Meaning of "percent parasitism" in studies of insect parasitoids. *Environmental Entomology* **12:** 1611-1622.

van Driesche, R. G. (1988a). Field measurement of population recruitment of *Apanteles glomerata* (L.) (Hymenoptera: Braconidae), a parasitoid of *pieris rapae* (L.) (Lepidoptera: Pieridae), and factors influencing adult parasitoid foraging success in kale. *Bulletin of Entomological Research* **78:** 199-208.

van Driesche, R. G. (1988b). Survivorship patterns of larvae of *pieris rapae* (L.) (Lepidoptera: Pieridae) in Massachusetts kale, with special reference to mortality due to *Apanteles glomerata* (L.) (Hymenoptera: Braconidae). *Bulletin of Entomological Reseach* **78:** 397-405.

van Driesche, R. G. and Bellows, T. S., Jr. (1988). Host and parasitoid recruitment for quantifying losses from parasitism, with reference to *Pieris rapae* and *Cotesia glomerata*. *Ecological Entomology* **13:** 215-222.

van Driesche, R. G., Bellows, T. S., Jr., Elkinton, J. S., Gould, J. R. and Ferro, D. N. (1991). The meaning of percentage parasitism revisited: solutions to the problem of accurately estimating total losses from parasitism. *Environmental Entomology* **20:** 1-7.

van Driesche, R. G., Bellows, T. S., Jr., Ferro, D. N., Hazzard, R. and Maher, A. (1989). Estimating stage survival from recruitment and density data, with reference to egg mortality in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *The Canadian Entomologist* **121:** 291-300.

van Lenteren, J. C. (1976). The development of host discrimination and the prevention of super-parasitsm in the parasite *Pseudeucoila bochei* Weld (Hym.:Cynipidae). *Netherlands Jounal of Zoology* **26:** 1-83.

Velasco, L. R. I. (1982). The life history of *Apanteles plutellae* Kurdj. (Braconidae), a parasitoid of the diamondback moth. *The Philippine Entomologist* **5:** 385-399.

Venkatraman, T. V. (1964). Experimental studies in superparasitism and multiparasitism in *Horogenes cerophaga* (Grav.) and *Hymenobosmina rapi* (Cam.), the larval parasites of *Plutella maculipennis* (Curt.). *Indian Journal of Entomology* **26:** 1-32.

Verkerk, R. H. J. and Wright, D. J. (1994). Interactions between the diamondback moth, *Plutella xylostella* L. and glasshouse and outdoor-grown cabbage cultivars. *Annals of Applied Biology* **125:** 477-488.

Vet, L. E. M. and van Opzeeland, K. (1985). Olfactory microhabitat selection in *Leptopilina heterotoma* (Thompson) (Hym : Eucoilidae), a parasitoid of Drosophilidae. *Netherlands Journal of Zoology* **35:** 497-504.

Vet, L. E. M., Datema, A., van Welzen, K. and Snellen, H. (1993). Clutch size in a larval-puapl endoparasitoid. 1. Variation across and within host species. *Oecologia* **95:** 410-415.

Vinson, S. B. (1976). Host selection by insect parasitoids. *Annual Review of Entomology* **21:** 109-133.

Vinson, S. B. (1981). Habitat location. *Semio chemicals: their role in pest control* (ed. by D.A. Nordlund, R.L. Jones and W.J. Lewis), pp. 51-77. John Wiley and Sons, New York.

Vinson, S. B. (1984a). Parasitoid-host relationship. *Chemical Ecology of Insects* (ed. by W.J. Bell and R.T. Carde), pp. 205-233. Chapman and Hall, London.

Vinson, S. B. (1984b). How parasitoids locate their hosts: a case of insect espionage. *Insect Communication* (ed. by T. Lewis), pp. 325-348. Academic Press Inc., London.

Vinson, S. B. and Iwantsch, G. F. (1980). Host suitability for insect parasitoids. *Annual Review of Entomology* **25:** 397-419.

Vos, H. C. C. A. A. (1953). Introduction in Indonesia of *Agnitia cerophaga* Grav., a parasite of *Plutella maculipennis* (Curt.). *Contribution of the General Agricultural Research Station, Bogor* **134:** 32 pp.

Waage, J. K. (1982). Sex ratio and population dynamics of natural enemies-some possible interactions. *Annals of Applied Biology* **101:** 159-164.

Waage, J. K. (1992). Biological control in the year 2000. *Pest management and the environment in 2000* (ed. by A. Aziz, S.A. Kadir and H.S. Barlow), pp. 329-340. C. A. B. International, U.K.

Waage, J. K. and Cherry, A. (1992). Quantifying the impact of parasitoids on diamondback moth. *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop* (ed. by N.S. Talekar), pp. 245-253. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Waage, J. K. and Hassell, M. P. (1982). Parasitoids as biological control agents- a fundamental approach. *Parasitology* **84:** 241-268.

Waage, J. K. and Mills, N. J. (1992). Biological control. *Natural enemies, the population biology of predators and diseases* (ed. by M.J. Crawley), pp. 412-430. Blackwell Scientific Pub, Oxford, London.

Wakisaka, S., Tsukuda, R. and Nakasuji, F. (1992). Effects of natural enemies, rainfall, temperature and host plants on survival and reproduction of the diamondback moth. *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop* (ed. by N.S. Talekar), pp. 15-26. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Waterhouse, D. F. (1992). Biological control of diamondback moth in the Pacific. *Diamondback moth and other crucifer pests : Proceedings of the Second International workshop* (ed. by N.S. Talekar), pp. 213-223. Asian Vegetable Research and Development Centre, Tainan, Taiwan.

Waterhouse, D. F. (1993). *The major arthropod pests and weeds of agriculture in Southeast Asia: distribution, importance and origin.* ACIAR, Canberra.

Waterhouse, D. F. and Norris, K. R. (1987). *Biological control pacific prospects.* Inkata Press, Melbourne.

Way, M. J. and Heathcote, G. D. (1966). Interactions of crop density of field beans, abundance of *Aphis fabae*, virus incidence and aphid control by chemicals. *Annals of Applied Biology* **57:** 409-423.

Wedding, R., Anderbrant, O. and Jonsson, P. (1995). Influence of wind conditions and intertrap spacing on pheromone trap catches of male European pine sawfly, *Neodiprion sertifer*. *Entomologia Experimentalis et Applicata* **77:** 223-232.

Weisser, W. W. (1994). Age-dependent foraging behaviour and host-instar preference of the aphid parasitoid *Lysiphlebus cardui*. *Entomologia Experimentalis et Applicata* **70:** 1-10.

Weseloh, R. M. (1976). Reduced effectiveness of the gypsy moth parasite, *Apanteles melanoscelus*, in Connecticut due to poor seasonal synchronization with its host. *Environmental Entomology* **5:** 743-746.

White, J. and Harper, J. L. (1970). Correlated changes in plant size and number in plant populations. *Ecology* **58:** 467-485.

Wiklund, C. (1977). Oviposition, feeding and spatial separation of breeding and foraging habitats in a population of *Leptidea sinapis*. *Oikos* **28:** 56-68.

Wilding, J. L., Barnett, A. G. and Amor, R. L. (1986). *Crop Weeds.* Inkata Press, Melbourne.

Wilson, F. (1960). A review of the biological control of insects and weeds in Australia and Australian New Guinea. *Commonwealth Institute of Biological control. Technical Communication* No. 1.

Wolfson, J. L. (1980). Oviposition response of *Pieris rapae* to environmentally induced variation in *Brassica nigra*. *Entomologia Experimentalis et Applicata* **27:** 223-232.

Wong, T. T. Y., Ramadan, M. M., McInnis, D. O. and Mochizuki, N. (1990). Influence of cohort age and host age on ovipostion activity and offspring sex ratio of *Biosteres tryoni*

(Hymenoptera: Braconidae), a larval parasitoid of *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Economic Entomology* **83:** 779-783.

Yamada, H. (1983). Development and fecundity of the diamondback moth on some cruciferous weeds. *Proceedings Kansai Plant Protection Society* **25:** 53 (in Japanese).

Yamada, H. and Umeya, K. (1972). Seasonal changes in wing length and fecundity of the diamondback moth, *Plutella xylostella* (L.). *Japanes Journal of Applied Entomology and Zoology* **16:** 180-186. (In Japanese with English summary).

Yang, J. C., Chu, Y. I. and Talekar, N. S. (1993). Biological studies of *Diadegma semiclausum* (Hym., Ichneumonidae), a parasite of diamondback moth. *Entomophaga* **38:** 579-586.

Yano, S. and Ohsaki, N. (1993). The phenology and intrinsic quality of wild crucifers that determine the community structure of their herbivorous insects. *Researches on Population Ecology* **35:** 151-170.

Yarrow, W. H. T. (1970). Parasites of *Plutella xylostella* (L.) in south-eastern Queenslan. *Queensland Journal of Agricultural and Animal Science* **27:** 321-324.

Zar, J. H. (1984). *Biostatistical analysis.* Prentice-Hall International, London.