Impact of the Microbial Pesticide *Bacillus thuringiensis* Berliner subsp. *kurstaki* on Hymenopterous Parasites of the Imported Cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae).

by

Richard Carlisle McDonald

Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Entomology

APPROVED:

L.T. Kok, Chairman

J.L. Eaton

R.L. Pienkowski

C.R. O'Dell

F.W. Ravlin

A.A. Yousten
Impact of the Microbial Pesticide *Bacillus thuringiensis* Berliner subsp. *kurstaki* on Hymenopterous Parasites of the Imported Cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae).

by

Richard Carlisle McDonald

Abstract

Three formulations of *Bacillus thuringiensis* Berliner subsp. *kurstaki* (Dipel 4L, Dipel 2X, and ABG-6167) were compared with the synthetic pyrethroid permethrin (common name Pounce 3.2 EC) for insecticidal activity and impact upon parasitism of the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae) in field broccoli (CV Packman) from fall 1986 to spring 1988. Permethrin, Dipel 4L, and ABG-6167 were not significantly different in their efficacy towards imported cabbageworm larvae.

Parasitization of *P. rapae* by the larval parasite *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) and the pupal parasite *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) continued after spraying in the *B. thuringiensis* plots, but was not significantly different from permethrin. However, 100% of the *P. rapae* chrysalids recovered were parasitized by *P. puparum*.

The impact of seasonal hyperparasitism was compared between *C. glomerata*, and a Yugoslavian strain of *Cotesia rubecula* (Marshall), an exotic larval parasite of the
imported cabbageworm. Four hyperparasites, two of them attacking both C. glomerata and C. rubecula, were found in field plots from 1986-1988. The level of hyperparasitization for the two primary parasites was significantly different. Hyperparasitization averaged only 8.1% for C. glomerata from 1986-1988, but was 37.9% for C. rubecula from 1987-1988. During the early- to mid-season of 1988, hyperparasite activity was not detectable and C. rubecula outcompeted C. glomerata for hosts; but by mid-season, hyperparasite activity against C. rubecula increased to 100%, causing its populations to crash. C. glomerata then became the dominant parasite of P. rapae. C. rubecula was not recovered in 1989. Hyperparasites may be a limiting factor in establishing C. rubecula in southwestern Virginia.

Mortality and successful pupation of P. rapae fourth instars parasitized by C. rubecula to B. thuringiensis endotoxin at dosages of 850, 85, and 8.5 I.U./ml was examined. After day two, the LC50’s of parasitized fourth instars were approximately thirty times higher than that of unparasitized larvae and by day four, the LC50 response of parasitized fourth instars was 180 times higher than unparasitized larvae. Twenty-five percent of parasitized fourth instars exposed to a concentration of 850 I.U./ml successfully pupated, compared to 76% at 85 I.U./ml and 69% at 8.5 I.U./ml. Parasitized fourth instar P. rapae consume less food and are therefore less susceptible to B. thuringiensis than unparasitized larvae at the same dosages.
I dedicate this dissertation to my father, the late Isaac Henry McDonald (the only Irish Jew in captivity), who taught me the values of hard work, patriotism, and pride in yourself; and to my mother, Selma Bloess Norton, who instilled in me at a young age a love of nature; with it, life can never be long enough...
Acknowledgements

Sometimes to say thank you is to cheapen the gift. The gift is that little extra which doesn’t need to be given, but that proper spirit and attitude transmit. I thank the Department of Entomology at VPI & SU for the gift of an excellent learning environment, created by its faculty and staff.

It is difficult for me to properly acknowledge on paper the help, insight, and philosophies I have been exposed to during my graduate career from my advisor, Dr. L.T. Kok. I am deeply indebted to him for financial assistance, facilities, understanding, and encouragement he provided throughout the course of this study. He has unselfishly shared his outlooks and experiences with me, and set an example which has helped guide me towards true scientific enlightenment. I deeply appreciate and respect his help.

I would also like to thank the members of my Ph.D. committee for their help during the course of this study and for their critical reviews and helpful suggestions in writing this dissertation: Dr. J. L. Eaton, for sharing his philosophies on education and science; C.R. O’Dell for his practical advice and extension experience; Dr. R.L. Pienkowski for ecological and entomological knowledge and teaching skills; Dr. F.W. Ravlin for his expertise in computing and statistics; and Dr. A.A. Yousten for laboratory space and microbiological techniques.
Ben Puttler, USDA Collaborator and co-advisor for my Master’s degree at the University of Missouri, provided me with the Yugoslavian strain of *Cotesia rubecula* (Marshall) and with tremendous insight into the field of biological control. His many helpful ideas and suggestions made many parts of this study possible. I am especially grateful for his continued help and friendship.

Many thanks go out to Warren Mays and Tom McAvoy for their field expertise and assistance in planting broccoli under the hot summer sun. Thanks also to Karen Vail, Rey Abad, Ban Na Ang and David Gaines for their assistance. I would also like to thank everyone who me helped plant broccoli: Rey Abad, my wife Kathryn Cahow, Colleen Cannon, Mark Carter, Deb Davidson, Dr. R. Fell, Ruying Feng, Holly Ferguson, Jim Harmon, Lorraine Koller (4-time champ!), Ray Layton, Warren Mays, Tom McAvoy, Hamid Norowi, Ken Stein, Mark Wooster, and the farm crew at the VPI & SU Horticulture Research Farm. I’d also like to thank Horticulture Farm Manager John Wooge for his assistance throughout the course of my field studies.

Specific individuals helped in different phases of data analyses. Shelby Fleischer’s knowledge of statistics and datafile creation was invaluable. Likewise, Keith Tignor’s advice and help in data analysis was much appreciated. Dr. M.L. Lentner of the Statistics Department helped to keep my statistical "feet" on the ground during moments of confusion.
I would like to thank the members and donors of the David R. Spence Scholarship for honoring me with their award. The Virginia Agricultural Council Project also provided partial funding.

Dr. R.D. Fell has been a special friend and workout partner. I've always gotten a big "kick" out of exercising with him!

Lastly, my wife has been a continual source of encouragement during the course of this study. She has helped me in late night hours taking care of insects at the insectary; she has also been alone through many nights as I worked late. Without her, I wouldn't have been able to accomplish what I have. Kathryn Cahow, I love you!
Table of Contents

Abstract .......................................................... ii
Acknowledgements ................................................... v

Chapter

Introduction ......................................................... 1

Chapter 1 - Literature Review .................................... 4

Chapter 2 - Comparative Efficacy of Three Formulations of *Bacillus thuringiensis* Berliner subsp. kurstaki and Their Effect on Parasitization of *Pieris rapae* (L.) (Lepidoptera: Pieridae) in Broccoli. .......... 19

Introduction ........................................................ 20
Materials and Methods ............................................. 21
Results and Discussion ........................................... 24
Fall 1986 .............................................................. 24
Spring 1987 Part A .................................................... 31
Spring 1987 Part B .................................................... 35
Fall 1987 .............................................................. 39
Spring 1988 ............................................................ 44

Chapter 3 - Intra-Plant Distribution of the Imported CabbageWorm, Diamondback Moth, and Their Parasites on Broccoli. ........... 55

Introduction ........................................................ 56
Materials and Methods ............................................. 59
Results and Discussion .......................................... 60
Diamondback Moth ................................................ 60
Imported CabbageWorm ............................................. 60

Chapter 4 - Cold Storage of *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) Prepupae in Host Chrysalid. ......................... 65

Introduction ........................................................ 66
Materials and Methods ............................................. 67
Results and Discussion .......................................... 68
Chapter 5 - Seasonal Hyperparasitism of *Cotesia glomerata* (L.) and *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) in Southwestern Virginia. 72

Introduction. 73
Materials and Methods. 75
Results and Discussion. 77
*Cotesia glomerata*. 77
*Cotesia rubecula*. 82

Chapter 6 - Susceptibility of *Pieris rapae* (L.) Larvae Parasitized by the Braconid *Cotesia rubecula* (Marshall) to Sublethal Dosages of *Bacillus thuringiensis* Berliner subsp. *kurstaki*. 92

Introduction. 93
Materials and Methods. 95
Results and Discussion. 96

Summary. 101

Literature Cited. 105

Appendix. 113

Vita. 121
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mean number of imported cabbageworm larvae per plant 3 days after the first spray application for fall 1986.</td>
<td>32</td>
</tr>
<tr>
<td>2. Mean seasonal parasitization rates of <em>P. rapae</em> larvae by treatment.</td>
<td>32</td>
</tr>
<tr>
<td>3. Mean imported cabbageworm larvae after spray treatments in Part A during spring 1987.</td>
<td>38</td>
</tr>
<tr>
<td>4. Mean imported cabbageworm larvae 5 days after initial spray in Experiment B spring 1987.</td>
<td>43</td>
</tr>
<tr>
<td>5. Mean number of imported cabbageworm larvae (instars 1-5) 3 days after spraying, fall 1987.</td>
<td>47</td>
</tr>
<tr>
<td>6. Mean number of imported cabbageworm larvae on 7 July 1988, 5 days after spraying.</td>
<td>48</td>
</tr>
<tr>
<td>7. Occurrence of <em>P. rapae</em> and <em>P. xylostella</em> life stages by position on the broccoli plant for fall 1986 and spring 1987.</td>
<td>61</td>
</tr>
<tr>
<td>8. Mean number of <em>P. puparum</em> adults emerging per chrysalid after storage at 10°C.</td>
<td>69</td>
</tr>
<tr>
<td>9. Mean percentage of hyperparasites recovered from <em>C. glomerata</em> cocoons across all plots from 1986-1988.</td>
<td>79</td>
</tr>
<tr>
<td>10. Mean percentage of hyperparasites recovered from <em>C. rubecula</em> cocoons across all plots from 1987-1988.</td>
<td>84</td>
</tr>
<tr>
<td>12. Response of fourth instar <em>P. rapae</em> larvae parasitized by <em>C. rubecula</em> compared to unparasitized fourth instars.</td>
<td>98</td>
</tr>
<tr>
<td>13. Summary of <em>C. rubecula</em> colony rearing from June 1987 to March 1989.</td>
<td>120</td>
</tr>
</tbody>
</table>
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mean <em>P. rapae</em> eggs per 40 plants for fall 1986</td>
<td>25</td>
</tr>
<tr>
<td>2. Mean <em>P. rapae</em> small larvae per 40 plants for fall 1986</td>
<td>26</td>
</tr>
<tr>
<td>3. Mean large <em>P. rapae</em> larvae per 40 plants for fall 1986</td>
<td>28</td>
</tr>
<tr>
<td>4. Mean <em>C. glomerata</em> cocoon masses per 40 plants for fall 1986</td>
<td>29</td>
</tr>
<tr>
<td>5. Mean <em>P. puparum</em>-parasitized <em>P. rapae</em> chrysalids per 40 plants for fall 1986</td>
<td>30</td>
</tr>
<tr>
<td>6. Mean <em>P. rapae</em> eggs for spring 1987 part A</td>
<td>34</td>
</tr>
<tr>
<td>7. Mean <em>P. rapae</em> small larvae for spring 1987 part A</td>
<td>36</td>
</tr>
<tr>
<td>8. Mean <em>P. rapae</em> large larvae for spring 1987 part A</td>
<td>37</td>
</tr>
<tr>
<td>9. Mean <em>P. rapae</em> eggs per plant for spring 1987 part B</td>
<td>40</td>
</tr>
<tr>
<td>10. Mean <em>P. rapae</em> small larvae for spring 1987 part B</td>
<td>41</td>
</tr>
<tr>
<td>11. Mean <em>P. rapae</em> large larvae for spring 1987 part B</td>
<td>42</td>
</tr>
<tr>
<td>12. Mean <em>P. rapae</em> total instars for fall 1987</td>
<td>45</td>
</tr>
<tr>
<td>13. Ovipositional activity by <em>P. rapae</em> for spring 1988</td>
<td>47</td>
</tr>
<tr>
<td>14. Mean small <em>P. rapae</em> larvae for spring 1988</td>
<td>49</td>
</tr>
<tr>
<td>15. Mean large <em>P. rapae</em> larvae for spring 1988</td>
<td>50</td>
</tr>
<tr>
<td>16. Mean <em>C. glomerata</em> cocoon masses for spring 1988</td>
<td>51</td>
</tr>
<tr>
<td>17. Mean <em>P. rapae</em> chrysalids parasitized by <em>P. puparum</em> for spring 1988</td>
<td>52</td>
</tr>
<tr>
<td>18. Relationships between <em>P. rapae</em>, its larval parasites <em>C. glomerata</em> and <em>C. rubecula</em>, and their respective hyperparasites</td>
<td>78</td>
</tr>
</tbody>
</table>
19. Hyperparasitization of *C. glomerata* cocoon samples from 1986-1988 . . . . . . . . . . . . . 81

20. Hyperparasitization of *C. rubecula* cocoon samples for 1987-1988. . . . . . . . . . . . . 84


22. Percentage pupation of *C. rubecula* in *B. thuringiensis*-intoxicated 4th instar larvae. . . 99
Introduction

Due to projected declines in both acreage and profitability of tobacco in the early 1980's, tobacco farmers in the southern Piedmont of Virginia began experimenting with broccoli as an alternate crop. Broccoli production in Virginia rose from 20 acres in 1983 to 800 acres in 1986. An early freeze killed the majority of the 500 acres of broccoli planted in 1987. Coupled with a rebound of tobacco due to new export markets, little broccoli was grown in 1988. In 1989, only 100 acres of broccoli was grown. Concurrently, tobacco acreages increased 19% in 1989, again due to strong export markets. However, with the establishment of regional truck crop markets in Virginia, a larger East Coast demand for broccoli than supply, and an uncertain long term future for tobacco, the outlook for continued broccoli production is good.

Currently, no integrated pest management program exists for the control of lepidopterous pests in broccoli. Studies conducted at VPI & SU from 1981 to 1989 have shown the imported cabbageworm, \textit{Pieris rapae} (L.) (Lepidoptera: Pieridae) to be one of the predominant injurious pest species on cruciferous crops in Virginia. These studies have also shown two hymenopterous parasites cause a significant amount of mortality in the imported cabbageworm. The two parasites are \textit{Cotesia glomerata} (L.) (Hymenoptera:
Braconidae), a gregarious larval parasite which averages 25% seasonal parasitization, and *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) a gregarious pupal parasite which averages over 50% seasonal parasitization. Although *C. glomerata* was introduced into the United States in 1883 to reduce imported cabbageworm populations and is one of its most common larval parasites, it does not suppress populations of *P. rapae* below damaging levels.

Since *P. rapae* is native to Europe, its complement of specific natural enemies may be lacking in North America. In the Palearctic region, the most common larval parasite of *P. rapae* is *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae). *C. rubecula* is a solitary endoparasite of *P. rapae* larvae. In the United States, the major larval parasite of *P. rapae* is *C. glomerata*.

A research effort was undertaken to enhance the natural enemy complex attacking *P. rapae* in Virginia. Three major objectives were pursued. First, permethrin and three formulations of *Bacillus thuringiensis* Berliner subsp. *kurstaki* were compared in the field for their insecticidal efficacy and impact on parasitism of the imported cabbageworm in broccoli. The natural enemy complex in crucifers could be enhanced by using *B. thuringiensis*, which is toxic to lepidopterous larvae but not to the adult parasites. Second, a Yugoslavian strain of *C. rubecula* was obtained and its potential for enhanced biocontrol of *P. rapae* was investigated. Third, laboratory studies assessed
the sublethal effects of *B. thuringiensis* to fourth instar *P. rapae* parasitized by *C. rubecula*.

The overall goal of this research was to provide data upon which Virginia's broccoli growers could establish a sound integrated pest management program, using conservation of natural enemies and selective microbial pesticides as the cornerstones of pest management options.
Chapter 1
Review of Literature

Broccoli, *Brassica oleracea* var. *italica* Plenck, is a cruciferous vegetable forming a short erect stem which produces a large green head of abortive succulent flowers. Broccoli, cabbage and the other cultivars of *Brassica oleracea* evolved from the colewort, a stout, weedy perennial of the seacoasts of Great Britain and southwestern Europe (Hill 1952).

Broccoli has been cultivated for thousands of years. A form of broccoli was known to the Greeks and Romans (Hill 1952). The present day broccoli was developed by the Italians in the Middle Ages from bunched kale (Haughton 1978).

In 1985, broccoli production in the U.S.A. totaled 42,560 ha (106,400 acres) (Agricultural Statistics Index 1986) with the following breakdown by states: California, 37,769 ha (94,400 acres), Texas, 3,040 ha (7,600 acres), Oregon, 1,000 ha (2,500 acres), and Arizona, 760 ha (1,900 acres). The crop had a total value of $231,468,000.

Due to declining domestic consumption, planted acreage and profitability of tobacco, farmers in the Piedmont area of southern Virginia began turning to broccoli in 1983 to supplement their income (O'Dell et al. 1989a, Fultz 1986). Broccoli production in Virginia rose from 20 acres in 1983 to 800 acres in 1986 (Vail & Kok 1989). The appeal of
broccoli to many tobacco growers lies in the fact that machinery used to plant and culture tobacco can readily be used to produce broccoli. Broccoli, like tobacco, can be sown in seed beds and later transplanted in the field, or be directly field seeded for greater efficiency where terrain allows (O’Dell et al. 1989b). Broccoli must also be irrigated periodically and most tobacco producers have irrigation systems.

In 1985, tobacco farmers in the southern Piedmont area of Virginia harvested approximately 300 acres of broccoli, receiving $6.17 per box after all marketing costs. Each box contained an average of 18 broccoli bunches. Farmers averaged $500 net return above all labor and marketing costs per acre of broccoli planted, although $1000 per acre or more is possible (Fultz 1986).

Since broccoli is an introduced vegetable, most of its insect pests originated in Europe or are polyphagous species native to the U.S. [e.g., Trichoplusia ni (Hübner)] (Bonnemaison 1965). The major lepidopterous pest complex of crucifers in the United States generally consists of the cabbage looper, T. ni (Hübner) (Lepidoptera: Noctuidae), the imported cabbageworm, Pieris rapae (L.) (Lepidoptera: Pieridae), and the diamondback moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae). The rank of species importance generally varies with latitude. In the more southern areas of the U.S.A., T. ni ranks as the primary pest, followed by P. rapae and P. xylostella (Smith &

The biology of P. rapae, P. xylostella, and T. ni in southwestern Virginia has been studied in detail (Chamberlin & Kok 1986, Lasota & Kok 1986a, Lasota & Kok 1986b). P. rapae and T. ni were the predominant injurious species of unprotected cabbage in southwest Virginia in 1981 and 1982 (Chamberlin & Kok 1986); P. rapae was the major pest of unprotected cabbage in this same area from 1982 to 1984 (Lasota & Kok 1989). T. ni was the most injurious lepidopterous pest of broccoli in the southern Piedmont area of Virginia in 1985 & 1986 (McAvoy et al. 1986) and the cabbage webworm Hellula rogatalis (Hulst) (Lepidoptera: Pyralidae), was most abundant in 1987 (Kok & McAvoy 1989).

The following is a brief overview of the life histories of P. rapae, P. xylostella, and their parasites.

**Life Histories**

**Pieris rapae** (L.) (Lepidoptera: Pieridae)

The imported cabbageworm, P. rapae, was first discovered in North America in 1860, when a single adult specimen was captured in Quebec (Harcourt 1963). Nearly thirty years later, P. rapae had spread north to Hudson’s Bay, south to the Gulf of Mexico, and west to the Rocky
Mountains. It now occurs throughout most of North America (Harcourt 1963).

*P. rapae* overwinters as a chrysalis in or near cruciferous crops. In Virginia, the adults emerge in March. Mating and oviposition occur within 24 hours of emergence (Harcourt 1963). Eggs are oviposited singly on the underside of the outer leaves of the host plant. Eclosion occurs in 4 to 8 days. The larvae pass through 5 instars in 12 to 33 days and then form a chrysalis. Adults emerge in 8 to 20 days and have a life span of approximately three weeks. Females *P. rapae* generally lay 200-300 eggs. In southwestern Virginia, the imported cabbageworm has 2-3 generations per year on cabbage (Chamberlin & Kok 1986, Lasota & Kok 1989). Courtney (1986) gave an excellent overview of the ecology of the Pieridae.

*Cotesia glomerata* (L.) (Hymenoptera: Braconidae)

*C. glomerata* (L.) (Mason 1981) (formerly *Apanteles glomeratus*) was the most common larval parasite of *P. rapae* in southwestern Virginia (Chamberlin & Kok 1986). It attacks the first three instars of *P. rapae*, ovipositing 20 to 50 eggs per host. Parasite larvae emerge en masse from the late fifth instar, and spin characteristic yellow cocoons. Parasitization by *C. glomerata* averaged 24.9% in 1981 and 24.3% in 1982, peaking at 40.0% in late August of 1981 and at 73.3% in late August of 1982 (Chamberlin & Kok 1986). Laing and Levin (1982) reviewed the biology and literature of *C. glomerata*. 
Pteromalus puparum (L.) (Hymenoptera: Pteromalidae)

P. puparum was accidentally introduced into the United States in the late 1800's (Oatman 1966). It is the most common parasite of P. rapae in southwestern Virginia. In surveys conducted from 1981-1984, parasitism of P. rapae by P. puparum in southwestern Virginia was greater than 50% (Chamberlin & Kok 1986, Lasota & Kok 1986a). Chamberlin and Kok (1986) observed peaks of 83.9% and 84.6% P. rapae chrysalids parasitized in late August 1981 and early August 1982, respectively. Lasota & Kok (1986a) observed peaks of 74.0% P. rapae chrysalids parasitized in early August 1983, and 84.0% in mid-August 1984.

P. puparum is a gregarious internal parasite of the imported cabbageworm, attacking the newly formed pupa (Clausen 1962). Larvae develop within the host and emerge as adults through a small hole cut in the pupal case. P. puparum colonies are often too large for the available food supply (Clausen 1962). Moss (1933) stated that numerous dead larvae were often found at the extremities of the pupa, having died from starvation. Lasota & Kok (1986a) observed continuous emergence of many adult parasites within a short period of time, although other authors reported a larval diapause of uncertain duration (Moss 1933, Clausen 1962). The mean number of individuals which develop in a single host pupa varies from 40.5 (Oatman 1966) to 52.3 (Lasota & Kok 1986a).
*Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

The diamondback moth, *P. xylostella*, is a cosmopolitan species, apparently originating from the southern Palearctic region bordering the Mediterranean Sea. It was first observed in North America by Fitch in the summer of 1854 near Ottawa, Illinois (Harcourt 1963). Further records of the moth did not appear in the literature until 1870, when damage was reported from Massachusetts, Maryland, and Michigan. By 1883, *P. xylostella* had spread south to Florida and west to the Rocky Mountains. It now occurs throughout the United States (Harcourt 1963).

The diamondback moth overwinters in the pupal stage, with adults emerging in early- to mid-May. Mating occurs at dusk on the day of emergence and lasts about one hour. Oviposition begins shortly after dusk and reaches its peak about two hours later. Eggs are oviposited singly or in small groups (two to eight), mainly on the upper surface of the host plant leaves (Harcourt 1963). Eclosion occurs in four to eight days. The larval period varies from 9 to 30 days, during which the larva passes through four instars. The mature larva constructs its cocoon typically on the lower leaves. Adults emerge in 5 to 15 days. The life span of the adult averages two weeks and during that time the female oviposits an average of 159 eggs. In Virginia, three generations of *P. xylostella* are known to occur on cabbage (Lasota & Kok 1986b); however, Harcourt (1963) reported four to six generations a year in Ontario. The potential for more
generations exists in areas of southwestern Virginia where cabbage is in continuous cultivation or wild cruciferae are present (Lasota & Kok 1989).

**Diadegma insulare** (Cresson)(Hymenoptera: Ichneumonidae)

*D. insulare* (=*insularis*), is the major parasite of *P. xylostella* in Virginia. The female attacks *P. xylostella* larvae, especially the later instars (Lasota & Kok 1986b). A solitary parasite emerges from the prepupa shortly after the host has spun its cocoon (Harcourt 1963). In southwestern Virginia, Lasota & Kok (1986b) reported seasonal parasitization of *P. xylostella* by *D. insulare* on cabbage to average 46.0% in 1983 and 69.0% in 1984.

Because the parasites of *P. rapae* and *P. xylostella* exert a significant amount of mortality upon their hosts, conservation and augmentation of these natural enemies would aid in developing an effective integrated control program for cruciferous crops in southern Virginia. The use of selective microbial insecticides to further reduce lepidopterous pest populations with minimal effect on natural enemies is one method of achieving natural enemy enhancement.

**Bacillus thuringiensis** Berliner

*B. thuringiensis* is a gram positive, spore-forming bacterium which is very pathogenic to a large number of lepidopterous larvae. Steinhaus (1963) reviewed the historical aspects and early work on the mode of action of crystalliferous bacteria. When *B. thuringiensis* sporulates,
it forms a crystal which is toxic when ingested by many lepidopterous larvae. Different Lepidoptera species exhibit varying responses when fed these crystals and/or *B. thuringiensis* spores (Heimpel & Angus 1959).

*P. rapae* is highly susceptible to *B. thuringiensis* in both the field and laboratory (Tanada 1953, Jaques 1972, Jaques & Morris 1981). Tanada (1953), using *B. thuringiensis* subsp. *berliner*, reported an LD$_{50}$ for fifth instars to be approximately 30,000 bacterial spores per larva, or about 450 spores per milligram of body weight.

**Impact of *B. thuringiensis* on parasites**

Although research has elucidated the effects of *B. thuringiensis* on lepidopterous larvae, more information concerning the impact of *B. thuringiensis* on parasites is needed. Jaques & Morris (1981) and Flexner et al. (1986) reviewed the compatibility of *B. thuringiensis* with non-target beneficial arthropods. Evidence indicates that *B. thuringiensis* may influence populations of parasitic species, but in general, much less than do chemical insecticides.

*P. rapae* larvae encounter and consume varying amounts of endotoxin and endospores on broccoli plants sprayed with *B. thuringiensis*. What effect this may have on parasitism is not well known. Weseloh & Andreadis (1982) hypothesized that the development of *B. thuringiensis*-intoxicated gypsy moth (*Lymantria dispar* (L.)) larvae may be retarded, in effect
extending the "ovipositional window" available to larval parasites. This hypothesis of synergism was later confirmed in the field (Weseloh et al. 1983).

B. thuringiensis-parasite interactions reported in the literature have been highly variable. The integration of B. thuringiensis and a braconid parasite (Cotesia melanoscela (Ratzeburg)) gave consistent suppression of gypsy moth larval populations (Wollam & Yendol 1976). Vail et al. (1972) found no parasitism of T. ni in B. thuringiensis-treated cabbage plots, although this could have been due to the small number of larvae collected. The addition of a sublethal dose of B. thuringiensis in diet fed to gypsy moth larvae increased the parasitism rates of the braconid Rogas lymantriae Watanabe (Wallner et al. 1983). However, sex ratios of the parasite progeny were altered, causing a reduction in the percentage of females as compared to the control. Kennedy & Oatman (1976) found a mixture of B. thuringiensis and Pirimicarb to have a minimal effect on the parasites of lepidopterous pests in broccoli.

Laigo & Paschke (1968) found that P. puparum adults reared from granulosis- and microsporidiosis-infected P. rapae chrysalids were generally smaller and shorter-lived than parasites reared from healthy hosts. Sex ratios were also adversely affected. Death of the infected host chrysalid before pupation of the parasite was the main source of P. puparum mortality. From histological
investigations, they attributed this to malnutrition of the P. puparum larvae and not to direct pathological effects.

It is doubtful that parasites and pathogens can be considered entirely compatible with each other because they are directly competing for the same host insect (Vail et al. 1972). A developing parasite in a diseased larva will not be able to complete development if the disease has progressed too far and the environment of the host becomes unsuitable for further development. Vail et al. (1972) suggested the two methods should not be used together unless the parasites are differentially effective on stages of development not in competition with B. thuringiensis, i.e., egg or pupa.

Commercial Production of Broccoli

Virginia Piedmont broccoli and southwestern Virginia cabbage growers commonly use a synthetic pyrethroid, permethrin (common names: Ambush or Pounce), to control lepidopterous larvae (Lasota & Kok 1986c). Permethrin is a "third generation" pyrethroid which appeared in 1973. Along with fenvalerate (common names: Pydrin or Asana), they became the first agricultural pyrethroids because of their exceptional insecticidal activity (0.11 kg AI/ha) and their photostability. Both are seemingly unaffected by ultraviolet rays in sunlight and last four to seven days on crop foliage as effective residues (Ware 1983). Virginia’s current recommendation for control of cabbage loopers and imported cabbageworms in broccoli was to treat with permethrin,
fenvalerate, and/or *B. thuringiensis* (Dipel) when an average of 0.5 larva per plant is present at head formation and development. Prior to head formation, higher levels of infestation are acceptable. (Virginia Cooperative Extension Service 1989).

Introduction of *Cotesia rubecula* for the control of *Pieris rapae*

Since *P. rapae* is an introduced pest, its complement of specific natural enemies may be lacking in North America (Blunck 1957). In the Palearctic region, the most common parasite of *P. rapae* is *Cotesia (=Apanteles) rubecula* (Marshall) (Richards 1941, Blunck 1957). In the United States, the major larval parasite of *P. rapae* is *C. glomerata* (Blunck 1957).

Parker (1970) found *C. glomerata* incapable of controlling *P. rapae* in Missouri for the following reasons: (1) the emergence of *C. glomerata* in the spring was asynchronous with that of its host, (2) *P. rapae* densities in the first 2 generations were too low to allow the parasites to increase at a rate which would allow them to suppress subsequent populations before economically important injury occurred, (3) Eggs of *C. glomerata* were frequently encapsulated when laid in late 2nd through 5th instar larvae.

*C. rubecula* has two biological attributes which suggest that it may have a greater potential in controlling *P. rapae*
than _C. glomerata_. First, in Europe, _C. rubecula_ is almost host specific to _P. rapae_, while _C. glomerata_ is primarily a parasite of _Pieris brassicae_ (L.) (Blunk 1957). Second, the solitary _C. rubecula_ normally exits from 4th-instar larvae; the gregarious _C. glomerata_ exits from late 5th-instar larvae (Puttler et al. 1970), which is the pest stage causing 85% of plant damage (Parker & Pinnell 1973).

_Cotesia rubecula_ (Marshall) (Hymenoptera: Braconidae)

_C. rubecula_ is a solitary larval endoparasite of the imported cabbageworm, _P. rapae_. It generally attacks the first three instars of _P. rapae_ larvae, although the first instar is preferred. Soon after the imported cabbageworm larva molts to the fourth instar, the _C. rubecula_ larva inside it exits and spins a white cocoon. Nealis (1985) gave a detailed account of the seasonal ecology of _C. rubecula_ in British Columbia.

The first North American record of _C. rubecula_ came from British Columbia in 1963 (Wilkinson 1966). How _C. rubecula_ became established there is unknown. Establishment of the parasite in Missouri and California from British Columbia stock proved unsuccessful (Oatman & Platner 1972, Nealis 1983). When transferred to Missouri, the _C. rubecula_ biotype from Vancouver continued to respond to a relatively high critical photoperiod, entering diapause in early September, even though the _P. rapae_ populations there continued to be active for several more months (Parker & Pinnell 1972). Nealis (1983) found that fall temperatures
averaging 15°C were lethal to diapausing *C. rubecula*. This explains the inability of the parasite to become established in Missouri and California.

Parker et al. (1971) and Laing & Corrigan (1987) found *C. rubecula* dominant when both *C. glomerata* and *C. rubecula* attack the same larvae. In numerous collections, when larvae of both species were found in the same larval host, *C. rubecula* had killed or was in the process of killing *C. glomerata*. Additionally, in fields where *C. rubecula* was released, *C. glomerata* failed to become an important parasite.

Hyperparasites of *C. glomerata* and *C. rubecula*

Hyperparasites have been recognized as an important facet in the effectiveness of biological control programs (Muesebeck & Dohanian 1927, Rosen 1981) and both *C. glomerata* and *C. rubecula* suffer from hyperparasitism (Richards 1941, Parker 1970, Nealis 1983). Blunk (1957) stated that hyperparasites in Europe "...are frequent and diminish the useful effect of *Apanteles glomeratus"*. Parker et al. (1971) found total hyperparasitization of *C. rubecula* introduced into Missouri varied from 36.6% to 72.4%. In British Columbia, Nealis (1983) reported 39.3% of *C. rubecula* cocoons were hyperparasitized.

*Catolaccus aeneoviridis* (Girault)(Hymenoptera: Pteromalidae)

*C. aeneoviridis* has been commonly recorded as both a primary and secondary solitary parasite on a wide range of

*Isdromas lycaenae* (Walker) (Hymenoptera: Ichneumonidae)

*I. lycaenae* is a solitary pupal hyperparasite of *Cotesia* (=Apanteles) spp., *Meteorus* spp., *Campoletis* spp., and *Hyposoter* spp. (Muesebeck et al. 1951).

*Spilochalcis torvina* (Cresson) (Hymenoptera: Chalcidoidea)

*S. torvina* is a solitary chalcid pupal endoparasite and has also been recorded as both a parasite and hyperparasite from a wide range of hosts (Arthur 1958, Burks 1940). Chittenden (1920) and Hough (1927) reported *Smicra* (=Spilochalcis) *torvina* in Virginia as a primary parasite attacking the red-banded leaf roller, Argyrotaenia velutinana (Walker).

*S. torvina* (as *S. side*) has been recorded in crucifer plantings as a primary parasite of *Ceutorhynchus assimilis* (Payk.) (Carlson et al. 1951, McLeod 1953), *P. xylostella* (Harding 1976a, McNeil & Rabb 1973), and *T. ni/Pseudoplusia includens* (Harding 1976b). *S. torvina* has also been recorded as a hyperparasite of the diamondback moth parasite, *D. insulare* (Marsh 1917, McNeil & Rabb 1973), and the imported cabbageworm larval parasite *C. glomerata* (McNeil & Rabb
1973, Parker 1970). S. torvina is currently listed as a synonym of S. side (Walker), but has been given species status by Couch (1984).

_Tetrastichus galactopus_ (Ratzeburg) (Hymenoptera: Eulophidae)

_T. galactopus_ is a gregarious eulophid endoparasite of braconid larvae, ovipositing directly into the parasite larvae through the body wall of the primary host. _T. galactopus_ appears to have entered North America with _C. glomerata_ which has been widely distributed for biological control of _P. rapae_ (Riley 1893, Nealis 1983). Nealis (1983) reviewed the biology and life history of _T. galactopus_.

The effects of hyperparasitism on populations of _C. rubecula_ or _C. glomerata_ have not been documented. Nealis (1983) believed that the persistence of _C. rubecula_ in Vancouver despite appreciable hyperparasitism by _T. galactopus_ argues against hyperparasitism as a predominant limitation to establishing _C. rubecula_ in North America.
Chapter 2

Comparative Efficacy of Permethrin and Three Formulations of Bacillus thuringiensis Berliner subsp. kurstaki and Their Effect on Parasitism of Pieris rapae (L.) (Lepidoptera: Pieridae) in Broccoli
Introduction

Historically, many entomological studies have dealt with the interactions between a pest insect and its complement of parasites. With the increasing use of microbial insecticides in the agricultural ecosystem (specifically Bacillus thuringiensis Berliner), a triad of complex interrelationships between the pest insect, its parasites, and the microbial pathogens which may infect the pest can be created. Although much research has been aimed at the effects of B. thuringiensis on lepidopterous larvae, more information concerning the impact of B. thuringiensis on parasites is needed. Jaques & Morris (1981) and Flexner et al. (1986) reviewed the compatibility of B. thuringiensis with non-target beneficial arthropods. Evidence indicates that B. thuringiensis may influence populations of parasitic species, but in general, much less than do chemical insecticides.

P. rapae is a major lepidopterous pest of crucifers in southwestern Virginia (Chamberlin & Kok 1986, Lasota & Kok 1989). It is attacked by a gregarious braconid larval parasite, Cotesia (=Apanteles) glomerata (L.), averaging 25% seasonal parasitization, and a gregarious pteromalid pupal parasite, Pteromalus puparum (L.), averaging over 50% seasonal parasitization (Chamberlin & Kok 1986, Lasota & Kok 1986a).

B. thuringiensis endotoxin must be ingested by lepidopterous larvae in order for the bacterial pesticide to
exert its toxic effect. Thus, coverage due to formulation is a major factor affecting the efficacy of *B. thuringiensis*. Three different formulations of *B. thuringiensis* were tested for their insecticidal efficacy and resultant impact on parasitism of the imported cabbageworm.

The objectives of this study were twofold: (1) To compare the efficacy of improved formulations of *B. thuringiensis* with the most commonly used synthetic pyrethroid (Pounce) for the control of *P. rapae*; and (2) To determine the impact of *B. thuringiensis* usage on parasitism of *P. rapae* in field broccoli.

**Materials and Methods**

Five treatments were used in this study: ABG-6158, an experimental oil emulsion of *B. thuringiensis* from Abbott Laboratories, now commercially available as Dipel 4L; ABG-6167, an experimental aqueous suspension of *B. thuringiensis* from Abbott Laboratories; a commercial formulation of Dipel 2X (wettable powder) from Abbott Laboratories; Pounce 3.2 EC (Permethrin), and a control sprayed only with water. These treatments were carried out in field plantings in fall 1986, spring and fall 1987, and spring 1988.

**Fall 1986.**

On 13 August 1986, 0.09 ha (18 by 50 m) of broccoli (Packman variety) was planted at the Virginia Polytechnic Institute and State University Horticultural Farm in a randomized complete block design having five treatments.
Each treatment consisted of three rows 10 m long. Plants were spaced 0.3 m apart, for a total of 30 plants per row. Rows were spaced 1 m apart with a 2 m gap between treatments. Treatments were replicated 4 times. Ten plants from the center row of each treatment were sampled twice weekly until frost killed the plants on 13 Nov. 1986. The incidence of *P. rapae* eggs, small larvae (instars 1-3), large larvae (instars 4-5), chrysalids, *C. glomerata* cocoons, and *P. rapae* chrysalids parasitized by *P. puparum* were recorded for each broccoli plant sampled.

A mean of 0.5 large larva per plant, the recommended action threshold for *P. rapae* in Virginia, was used to trigger the application of treatments in the plot. Each treatment was sprayed weekly for a total of three weeks. Treatments were applied at the rate of 4 B.I.U. (Billion International Units of *B. thuringiensis* endotoxin) per hectare for the three *B. thuringiensis* formulations. Pounce 3.2 EC was applied at the recommended rate of 33.3 ml/hectare.

The mean and variance of the each life stage of the imported cabbageworm sample data were plotted to determine their distribution. These data were found to have a Poisson (random) distribution. The data were then transformed using Square Root of (X+1). Analysis of variance and mean separations tests were performed among the treatments.
Spring 1987

For Spring 1987, the same experimental design, layout, and treatments as described earlier were used in fall 1986. However, the plot was divided into two parts (A and B) by halving the row length from ten meters to five meters. Both parts A and B were planted on 1 May 1987.

The spring 1987 experiment Part A was based on the same spray threshold values used in the fall 1986 study. Part B was designed to have weekly spray treatments once the initial larval action threshold was reached. Five plants from the center row of each treatment in part A and part B were sampled for the same life stages as in the fall 1986 study. These data were also found to have a Possion (random) distribution, so transformations and analyses were identical to those of fall 1986 data.

Fall 1987

For fall 1987, the broccoli study plot had to be relocated because the Horticultural Farm was sold. The broccoli plot was moved to the inner part of the Horticultural farm, surrounded by orchards on three sides and the former biology department arboretum on the west. The plot size and design were initially the same as that of fall 1986. The plot was planted on 24 August 1987. However, extensive groundhog damage necessitated the replanting of most of the plot on 2 September 1987 and the plot had to be scaled down to half the size of the fall 1986 plot (18 X 25 m). These data were also found to have a Possion (random)
distribution, so transformations and analyses were identical to those of fall 1986 data.

Spring 1988

On 26 April 1988, a plot of broccoli measuring 18 by 25 m was planted in the same location as the fall 1987 study. Treatments and plot design were also the same as in fall 1987. A border row of broccoli was planted around the plot and 15 P. rapae eggs were placed on each plant in order to increase the level of imported cabbageworms in the plot. A mean of 2.0 P. rapae larvae per plant was used to trigger the application of treatments. The higher action threshold of hosts was used so that parasites could increase before spraying. B. thuringiensis and Pounce treatments were applied at the same rate as in the fall 1986 study. These data were also found to have a Possion (random) distribution, so transformations and analyses were identical to those of fall 1986 study.

Results and Discussion

Fall 1986

The action threshold of 0.5 P. rapae larvae per plant was reached on 23 September; plants were sprayed on 26 September, 3 October and 10 October 1986. Ovipositional activity by P. rapae was not significantly different among the five treatments (Fig. 1). Most small larvae were making the transition to large larvae during the three spray dates indicated by arrows (Fig. 2). Counts of large larvae in the
Fig. 1. Mean P. rapae eggs per plant for fall 1986. Spray times are denoted by arrows (↓). n = 40 plants/treatment.
Fig. 2. Mean *P. rapae* instars 1-3 per plant for fall 1986. Spray times are denoted by arrows (†). n = 40 plants/treatment.
control treatment (Fig. 3) continued to increase after the first spray treatment and remained greater than 0.1 larva per plant through the middle of October.

Parasitism by *C. glomerata* occurred after spraying in the *B. thuringiensis* and control treatments (Fig. 4). *C. glomerata* cocoon masses were detected at most in three of the possible five treatments on any sample date after spraying. There was no parasitism observed in the Pounce treatment throughout the entire season. No hosts were available for *C. glomerata* to attack after the initial spraying in the Pounce-treated plots.

A *P. rapae* chrysalid was found on the first sample date after spraying in both the ABG-6167 and control treatments. Two more chrysalids were found in the control treatments on 24 October 1986. A pupa was found in the ABG-6167 treatments on 30 October and in the Dipel 2X plots on 30 October and 6 November. No chrysalids were recovered in the Pounce treatments during the entire season.

All *P. rapae* chrysalids found during the fall of 1986 were parasitized by *P. puparum* (Fig. 5). Parasitism by *P. puparum* continued after spraying in both the *B. thuringiensis* and control treatments.

Treatment means based on transformed data were not ranked differently from untransformed data in mean separation tests throughout this study, so the untransformed data are presented. A Duncan's mean separation for total imported cabbageworm larvae on 29 September (the first
Fig. 3. Mean *P. rapae* instars 4–5 per plant for fall 1986. Spray times are denoted by arrows (↓). *n* = 40 plants/treatment.
Fig. 4. Mean *C. glomerata* cocoon masses per plant for fall 1986. Spray times are denoted by arrows (▼). n = 40 plants/treatment.
Fig. 5. Mean *P. puparum*-parasitized *P. rapae* chrysalids per plant for fall 1986. Arrows (▼) are spray times. n = 40 plants/treatment.
sample date after the initial spraying) placed Pounce, Dipel 4L, and ABG-6167 in the same grouping (Table 1). There was no significant difference between the three *B. thuringiensis* formulations and none between Dipel 2X and the control.

The mean seasonal parasitization rates for *C. glomerata* and *P. puparum* are shown in Table 2. Parasitization rates for *C. glomerata* were two to seven times higher in the *B. thuringiensis*-treated plots than in the control plots. It is unclear whether this difference could have been due to a *B. thuringiensis*-induced slowing of the growth of *P. rapae* larvae which survived the treatments, thus extending the ovipositional window available to the parasites, or an anomaly due to low numbers of parasite cocoons masses recovered. The total number of parasite cocoon masses of *C. glomerata* was twelve.

For *P. puparum*, parasitization averaged 100% in all three *B. thuringiensis*-treated and control treatments. Larvae that pupated in the control and *B. thuringiensis* treatments were located and parasitized by *P. puparum*.

**Spring 1987 Part A**

A mean of 0.7 large larva/plant in the control plot on 15 June 1987 triggered spraying on 18 June, 28 June, and 6 July. The latter two spray dates were delayed by rain.

Data analysis was the same as for fall 1986 data. There were no significant differences in the number of *P. rapae* eggs laid among the five treatments before spraying (Fig. 6). However, after spraying was initiated, the Pounce plot
Table 1. Mean number of total imported cabbageworm larvae per plant 3 days after the first spray application for fall 1986. n = 40 plants/treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean(SE)</th>
<th>Duncan Grouping¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.600(0.127)</td>
<td>A</td>
</tr>
<tr>
<td>Dipel 2X</td>
<td>0.425(0.180)</td>
<td>A B</td>
</tr>
<tr>
<td>ABG-6167</td>
<td>0.225(0.075)</td>
<td>B C</td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>0.150(0.050)</td>
<td>B C</td>
</tr>
<tr>
<td>Pounce</td>
<td>0.025(0.025)</td>
<td>C</td>
</tr>
</tbody>
</table>

¹Means having the same letter are not significantly different, (P < 0.05), Duncan’s Multiple Range Test (1955).
Table 2. Mean seasonal parasitization rates of *P. rapae* larvae by treatment. For *C. glomerata*, the percentage of large larvae (fourth and fifth instars) parasitized was used to determine parasitization. n is the number of *C. glomerata* masses or *P. puparum*-parasitized host chrysalids.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Parasitization By</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. glomerata</em></td>
<td>n</td>
<td><em>P. puparum</em></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>ABG-6167</td>
<td>37.5</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>15</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Dipel 2X</td>
<td>13</td>
<td>3</td>
<td>100</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>3</td>
<td>100</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Pounce 3.2 EC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 6. Mean *P. rapae* eggs for spring 1987 part A. Arrows (▼) are spray times. n = 20 plants/treatment.
had significantly more ovipositional activity by *P. rapae*. Plants sprayed with Pounce had much less flea beetle damage, which made them more attractive as ovipositional sites to female *P. rapae*.

Seasonal counts of small (instars 1-3) and large (instars 4-5) larvae are presented in Figures 7 and 8, respectively. A Duncan’s mean separation test for total larvae (instars 1-5) on 22 June 1987 (first sample date after the initial spraying) showed that the control had significantly more larvae than the Dipel 2X, ABG-6167, or Pounce treatments (Table 3). Pounce was again the most efficacious insecticide, followed by ABG-6167, Dipel 2X, and Dipel 4L. After the first spray treatment, Dipel 4L was not as effective as the other *B. thuringiensis* treatments. However, by 30 June 1987 (Table 3), (the sample date after the second spraying), Dipel 4L was ranked second behind Pounce, which supports the findings of the fall 1986 study.

Parasitism was extremely low during the spring 1987 season. For part A, only two *C. glomerata* cocoon masses were found; one on 6 June in the Dipel 4L treatment and one on 21 July in the control. No parasitism by *P. puparum* was observed.

**Spring 1987 Part B**

A mean of 0.6 larva in the Pounce plot on 15 June 1987 triggered spraying on 18 June, 28 June, 6 July, and 15 July. The latter three spray dates were delayed by rain. Part B had only one more spray treatment than part A. Flea beetle
Fig. 7. Mean P. rapae small larvae for spring 1987
part A. Arrows (↑) are spray times.
n = 20 plants/treatment
Fig. 8. Mean *P. rapae* large larvae for spring 1987

part A. Arrows (▼) are spray times.

n = 20 plants/treatment
Table 3. Mean imported cabbageworm larvae (instars 1-5) per plant after spray treatments in part A spring 1987. n = 20 plants/treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean(SE)</th>
<th>Duncan Grouping(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4 days after initial spray</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.900(0.574)</td>
<td>A</td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>0.750(0.263)</td>
<td>A B</td>
</tr>
<tr>
<td>Dipel 2X</td>
<td>0.600(0.216)</td>
<td>B</td>
</tr>
<tr>
<td>ABG-6167</td>
<td>0.500(0.173)</td>
<td>B</td>
</tr>
<tr>
<td>Pounce</td>
<td>0.100(0.058)</td>
<td>B</td>
</tr>
<tr>
<td><strong>Two days after 2nd Spray</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.850(0.457)</td>
<td>A</td>
</tr>
<tr>
<td>Dipel 2X</td>
<td>0.450(0.222)</td>
<td>A B</td>
</tr>
<tr>
<td>ABG-6167</td>
<td>0.200(0.000)</td>
<td>B</td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>0.150(0.096)</td>
<td>B</td>
</tr>
<tr>
<td>Pounce</td>
<td>0.000(0.000)</td>
<td>B</td>
</tr>
</tbody>
</table>

\(^1\)Means having the same letter are not significantly different, (\(P < 0.05\)), Duncan's Multiple Range Test (1955).
damage and senescence of the plants prevented more sprays from being applied.

Figure 9 compares with Figure 6 in part A, showing that there were no significant differences in egg deposition among the five treatments before spraying. However, after spraying was initiated, the Pounce plot again had significantly more ovipositional activity by *P. rapae*.

Counts of small (instars 1-3) and large (instars 4-5) larvae throughout the season are presented in Figures 10 and 11, respectively. The control had significantly more imported cabbageworm larvae than the other treatments (Table 4). Pounce was again the most efficacious insecticide, followed by Dipel 4L, Dipel 2X, and ABG-6167. This ranking of Dipel 4L and Pounce is similar to results found in fall 1986, but different from the results in part A. However, both Dipel 4L and ABG-6167 were not significantly different from Pounce in terms of efficacy towards the imported cabbageworm.

Parasite activity in part B was also extremely low. Only one *C. glomerata* cocoon mass was found on 21 July in the control. A single *P. rapae* chrysalid which was found on 30 June was parasitized by *P. puparum*.

**Fall 1987**

Populations of *P. rapae* were extremely low throughout the season; the highest mean of *P. rapae* larvae was on 26 October in the ABG-6167 plot, when the mean number of larvae was only 0.3 per plant. The same count of 0.3 larva per
Fig. 9. Mean P. rapae eggs per plant for spring 1987 part B. Arrows (▼) are spray times.

n = 20 plants/treatment
Fig. 10. Mean *P. rapae* small larvae for spring 1987
part B. Arrows (↓) are spray times.

n = 20 plants/treatment
Fig. 11. Mean *P. rapae* large larvae for spring 1987 part B. Arrows (▼) are spray times.

\[ n = 20 \text{ plants/treatment} \]
Table 4. Mean number of imported cabbageworm larvae (instars 1-5) per plant five days after initial spray treatments in Part B spring 1987. n = 20 plants/treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean(SE)</th>
<th>Duncan Grouping(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.250(0.67)</td>
<td>A</td>
</tr>
<tr>
<td>ABG-6167</td>
<td>0.750(0.13)</td>
<td>B</td>
</tr>
<tr>
<td>Dipel 2X</td>
<td>0.550(0.42)</td>
<td>B</td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>0.400(0.14)</td>
<td>B</td>
</tr>
<tr>
<td>Pounce</td>
<td>0.200(0.08)</td>
<td>B</td>
</tr>
</tbody>
</table>

\(^1\)Means having the same letter are not significantly different, (P < 0.05), Duncan's Multiple Range Test (1955).
plant was reached on 2 November in the Dipel 2X plot. Figure 12 shows the mean number of *P. rapae* larvae of all instars throughout the growing season.

The broccoli plot was sprayed on 2 November, as the highest larval count was attained on this date and the end of the growing season was approaching. Due to the low numbers of *P. rapae*, none of the plots had significantly different larval counts after the spraying (Table 5). There was no parasite activity detected throughout the season.

**Spring 1988**

The action threshold of 2.0 larvae/plant was reached on 30 June 1988 and plants were sprayed on 2 July. Ovipositional activity by *P. rapae* was not significantly different among the five treatments (Fig. 13).

A mean separation for imported cabbageworm larvae (instars 1-5) on 7 July 1988 placed Pounce, Dipel 4L, and Dipel 2X in the same grouping (Table 6, Figs. 14 and 15). All treatments had significantly lower levels of insects than the control. This is consistent with results from the previous 3 seasons.

Parasite activity by *C. glomerata* and *P. puparum*, although not significant, occurred after spraying in both the *B. thuringiensis* and control treatments, but there was no parasite activity observed in the Pounce treatment throughout the entire season (Fig. 16 & 17). No hosts were available for the parasite to attack after the initial spraying in the Pounce-treated plots.
Fig. 12. Mean P. rapae total instars for fall 1987.

Arrow (↓) is the spray time.
n = 20 plants/treatment
Table 5. Mean number of imported cabbageworm larvae (instars 1-5) per plant 3 days after the first spray treatments in Fall 1987. n = 20 plants/treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean(SE)</th>
<th>Duncan Grouping¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipel 2X</td>
<td>0.175(0.061)</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>0.125(0.053)</td>
<td>A</td>
</tr>
<tr>
<td>ABG-6167</td>
<td>0.100(0.048)</td>
<td>A</td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>0.025(0.025)</td>
<td>A</td>
</tr>
<tr>
<td>Pounce</td>
<td>0.025(0.025)</td>
<td>A</td>
</tr>
</tbody>
</table>

¹Means having the same letter are not significantly different, (P < 0.05), Duncan's Multiple Range Test (1955).
Fig. 13. Ovipositional activity by *P. rapae* for spring 1988.

Spray time denoted by arrow (▼).

n = 20 plants/treatment
Table 6. Mean number of imported cabbageworm larvae (instars 1-5) per plant on 7 July 1988, 5 days after the initial spraying. n = 20 plants/treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean(SE)</th>
<th>Duncan Grouping(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.350(0.301)</td>
<td>A</td>
</tr>
<tr>
<td>ABG-6167</td>
<td>0.875(0.212)</td>
<td>B</td>
</tr>
<tr>
<td>Dipel 2X</td>
<td>0.625(0.132)</td>
<td>B C</td>
</tr>
<tr>
<td>Dipel 4L</td>
<td>0.325(0.090)</td>
<td>B C</td>
</tr>
<tr>
<td>Pounce</td>
<td>0.150(0.057)</td>
<td>C</td>
</tr>
</tbody>
</table>

\(^1\)Means having the same letter are not significantly different, (P < 0.05), Duncan's Multiple Range Test (1955).
Fig. 14. Mean small *P. rapae* larvae for spring 1988.
Spray time is denoted by arrow (▼).
n = 20 plants/treatment
Fig. 15. Mean large *P. rapae* for spring 1988.
Spray time is denoted by arrow (↓).
n = 20 plants/treatment
Fig. 16. Mean C. glomerata cocoon masses for spring 1988. Spray time is denoted by arrow (▼). n = 20 plants/treatment
Fig. 17. Mean *P. rapae* chrysalids parasitized by *P. puparum* for spring 1988. Spray time is an arrow (▼). n = 20 plants/treatment
After the Horticultural farm was sold, the fall 1987 and spring 1988 study plots had to be relocated to the interior of the farm, surrounded on three sides by orchards. Pesticides were intensively used in these orchards, and spray drift could have adversely affected the occurrence and number of *P. rapae* and its parasites in the broccoli plots.

Conclusions

Dipel 4L and ABG-6167 were not significantly different from Pounce 3.2 EC in terms of insecticidal efficacy towards *P. rapae* larvae over the 4 seasons tested.

The incidence of parasitism throughout this field study was too low for the treatments to be significantly different. However, parasite activity continued after spraying in the *B. thuringiensis*-treated and control plots in the fall 1986, spring 1987 and spring 1988 studies. Larval numbers of *P. rapae* were so low after the application of *B. thuringiensis* that parasitism by *C. glomerata* was never significant as compared to either the Pounce or control plots.

The parasitism of *P. rapae* chrysalids by *P. puparum*, although not significantly different from the Pounce treatments, was extremely high during the course of this study (100% of *P. rapae* chrysalids recovered). *P. puparum* shows promise for use in an integrated pest management scheme combined with Dipel 4L for the control of *P. rapae* larvae. I theorize that larvae which escape *B. thuringiensis*
treatment and pupate could have an increased chance of becoming parasitized by *P. puparum*, due to its high host finding capacity. This supports the conclusion of Vail et al. (1972) that the use of microbial pesticides with parasites which are not competing for the same life stage may be compatible.
Chapter 3

Intra-Plant Distribution of the Imported Cabbageworm, Diamondback Moth, and Their Parasites on Broccoli
Introduction

Larvae of the imported cabbageworm, Pieris rapae (L.) (Lepidoptera: Pieridae), and the diamondback moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae), are perennial pests of cruciferous crops in southwestern Virginia (Chamberlin & Kok 1986, Lasota & Kok 1989). The ability to predict the amount and location of damage within the plant caused by these two pests in broccoli is important in developing a pest management program for their control. If larval feeding is restricted to leaves, the damage is indirect, reducing the farmer’s profit only if it reduces broccoli head weight (Vail et al. 1989); if feeding is on the head, the crop can be rendered unmarketable, resulting in a total loss.

Previous studies in southwestern Virginia have shown that parasites exert a significant level of mortality upon populations of the imported cabbageworm and diamondback moth (Chamberlin & Kok 1986). The two major parasites attacking P. rapae are the gregarious larval parasite Cotesia glomerata (L.) (Hymenoptera: Braconidae) and the gregarious pupal parasite Pteromalus puparum (L.) (Hymenoptera: Pteromalidae). The major parasite attacking the diamondback moth is Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae).

C. glomerata attacks the first three instars of P. rapae, ovipositing 20 to 50 eggs in a host larvae. Parasite larvae remain in the host until the 5th instar, when they
emerge en masse and spin cocoons. Parasitization of imported cabbageworm larvae by *C. glomerata* averaged 24.9% in 1981 and 24.3% in 1982, peaking at 40.0% in late August of 1981 and at 73.3% in late August of 1982 (Chamberlin & Kok 1986).

*P. puparum* is a gregarious internal parasite of the imported cabbageworm, attacking the newly formed pupa (Clausen 1962). After completing development, adults emerge through a small hole cut in the pupal case. In surveys conducted from 1981-1984, parasitization of *P. rapae* by *P. puparum* in southwestern Virginia was greater than 50% (Chamberlin & Kok 1986, Lasota & Kok 1986a). Chamberlin and Kok (1986) observed peaks of 83.9% and 84.6% *P. rapae* chrysalids parasitized in late August 1981, and early August 1982, respectively. Lasota & Kok (1986a) observed peaks of 74.0% *P. rapae* chrysalids parasitized in early August 1983, and 84.0% in mid-August 1984.

*D. insulare* (*=insularis*), is a solitary larval parasite of *P. xylostella*. The female attacks *P. xylostella* larvae, especially the later instars (Lasota & Kok 1986b). A solitary parasite emerges from the prepupa shortly after the host has spun its cocoon (Harcourt 1963). In southwestern Virginia, Lasota & Kok (1986b) reported that seasonal parasitization of *P. xylostella* larvae by *D. insulare* on cabbage averaged 46.0% in 1983 and 69.0% in 1984.

Although several studies of insect distribution on cabbage have been conducted, information is lacking on the intra-plant distribution of the immature life stages of *P.*
**rapae, P. xylostella**, and their parasites on field broccoli.

Harcourt (1963) and Chalfant & Brett (1967) stated that the imported cabbageworm characteristically oviposited its eggs on the outer leaves of the cabbage plant. Lasota & Kok (1989) found that significantly more P. rapae eggs and small larvae (instars 1-3) were found on field cabbage frame leaves versus the head. In the laboratory, Hoy & Shelton (1987) found that late instar P. rapae preferred the youngest leaves of cabbage (i.e., head and wrapper leaves). In greenhouse studies, Samson & Geier (1983) found that third- through fifth-instar P. rapae larvae tended to feed on the upper leaves of cabbage plants. Because young cabbage leaves contain more nitrogen than old leaves (Samson & Geier 1983), P. rapae larvae may also adjust their rate of nitrogen intake by seeking leaves of a particular age (Hoy & Shelton 1987). Harcourt (1963) stated that mature P. xylostella larvae migrate in search of pupation sites and that the larva constructs its cocoon typically on the lower leaves of the cabbage plant.

Knowledge of areas of the broccoli plant which characteristically harbor greater numbers of specific life stages of P. rapae, P. xylostella, and their natural enemies can be of value when considering sampling plans for pest management and/or natural enemy conservation options. The objectives of this field study were to determine areas of the broccoli plant that characteristically harbor particular life stages of P. rapae, P. xylostella, and their parasites.
Materials and Methods

Fall 1986

On 13 August 1986, a plot of broccoli (Packman variety) 18 by 50 m was planted at the Virginia Polytechnic Institute and State University Horticultural Farm. Rows were spaced 1 m apart and plants were spaced 0.3 m apart in each row. Forty randomly selected plants were sampled twice weekly until harvest. The plants received no insecticidal treatments during the study. Starting with the uppermost leaf as leaf number one and progressing down the plant, the incidence of *P. rapae* eggs, small larvae (instars 1-3), large larvae (instars 4-5), *C. glomerata* cocoon masses, *P. puparum*-parasitized *P. rapae* chrysalids, *P. xylostella* larvae (all instars), pupae, and *D. insulare* cocoons were recorded on a per leaf basis for each plant sampled. *P. xylostella* eggs were not recorded due to their very small size.

Spring 1987

The same parameters were used for spring 1987 as in fall 1986. The spring 1987 broccoli plot was planted on 1 May 1987. Twenty randomly selected plants were sampled twice weekly until the the plants were harvested.

The per leaf data from fall 1986 and spring 1987 were combined for analysis. Leaf numbers were divided into three areas: upper (including the head), middle, and lower. A plot of the variance against the mean showed all life stages of
the data had a Poisson distribution. The data were transformed using Square Root of \((X+1)\). Analysis of variance and mean separations were used to determine differences among the three areas of the plant.

**Results and Discussion**

**Diamondback moth.** Significantly more *P. xylostella* larvae (all instars) were found in the middle leaves of the plant as compared to the upper and lower leaves (Table 7). *P. xylostella* females may selectively oviposit eggs on the upper leaves, but by the time of eclosion (4-8 days), new leaves forming would displace these leaves to the middle part of the plant.

No difference was observed for *P. xylostella* pupae or *D. insulare* cocoons in the three strata of the broccoli plant. These data suggest that fourth instar *P. xylostella* larvae are mobile and that both unparasitized and parasitized larvae seek out pupation sites randomly on the plant or in nearby vegetation. This supports Harcourt’s (1961) assertion that mature larvae migrate for short distances in search of suitable pupation sites, but differs from his observations in cabbage (Harcourt 1963) that *P. xylostella* larvae typically construct cocoons on the lower leaves.

**Imported cabbageworm.** The upper leaves of the broccoli plant contained significantly more *P. rapae* eggs than the middle leaves, and the middle leaves had significantly more eggs
Table 7. Occurrence of *P. rapae*, *P. xylostella* and their parasites by position on the broccoli plant for fall 1986 and spring 1987. Mean separation rankings of transformed data are given with untransformed means.

<table>
<thead>
<tr>
<th>Leaf position</th>
<th>P. rapae</th>
<th>Per 100 leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
<td>Small larvae</td>
</tr>
<tr>
<td>Upper¹</td>
<td>9.17ᵃ(0.76)</td>
<td>2.21ᵇ(0.37)</td>
</tr>
<tr>
<td>Middle²</td>
<td>4.27ᵇ(0.48)</td>
<td>3.27ᵃ(0.42)</td>
</tr>
<tr>
<td>Lower³</td>
<td>0.51ᶜ(0.24)</td>
<td>0.80ᶜ(0.26)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P. xylostella</th>
<th>Per 100 leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae</td>
</tr>
<tr>
<td>Upper¹</td>
<td>1.95ᵇ(0.32)</td>
</tr>
<tr>
<td>Middle²</td>
<td>4.49ᵃ(0.52)</td>
</tr>
<tr>
<td>Lower³</td>
<td>2.77ᵇ(0.52)</td>
</tr>
</tbody>
</table>

* Means within columns having the same letter are not significantly different (*P* < 0.05, Duncan's Multiple Range Test 1955). S.E.'s are in parentheses beside each mean.

¹ n = 1897 leaves  ² n = 1895 leaves  ³ n = 1370 leaves
than the lower leaves (Table 7). These data suggest that *P. rapae* females selectively oviposit in the upper portion of the broccoli plant which contains the younger leaves, but will also oviposit in the middle leaves. This is in contrast to cabbage, where *P. rapae* females preferentially oviposit on frame leaves rather than the head (Chalfant & Brett 1967, Lasota & Kok 1989). The difference in oviposition on broccoli and cabbage may be attributed to the behavior of the ovipositing *P. rapae* female, which prefers to oviposit on the underside of leaves (Lasota & Kok 1989) rather than on the upper surface of the exposed cabbage head.

The three strata of the broccoli plant were significantly different from each other in terms of the density of *P. rapae* small larvae. In descending order, small larvae were found in the middle, upper and lower parts of the plant. Upon eclosion, imported cabbageworm larvae lack mobility and are essentially confined to a single leaf until they reach the fourth instar. By the time that eggs oviposited on the upper leaves of the plant eclose, they are replaced through growth and become the middle leaves of the plant with first- through third-instar feeding on them.

The three strata of the broccoli plant were again significantly different from each other in terms of the density of *P. rapae* large larvae. In descending order, large larvae were found in the upper, middle, and lower parts of the plant. Since large larvae are mobile, the data suggest they migrate to the upper part of the plant, which contains
leaf material highest in nitrogen (Samson & Geier 1983). This supports Samson & Geier’s (1983) greenhouse studies and Hoy & Shelton’s (1987) laboratory studies showing that large *P. rapae* larvae (instars 4-5) prefer younger leaves of crucifers compared to older leaves. *C. glomerata* cocoons and *P. puparum*-parasitized *P. rapae* chrysalids were present in very low numbers and any meaningful conclusions about the distribution of these parasite life stages cannot be drawn.

Data from this study shows that the entire broccoli plant does not need to be sampled to obtain accurate population estimates of *P. rapae* and *P. xylostella*. As two-thirds of *P. rapae* eggs are oviposited in the upper leaves of the broccoli plant, and nearly sixty percent of large larvae occur in the upper leaves, sampling procedures based on leaves of the upper third of the plant could be used. By monitoring *P. rapae* eggs and large larvae in the upper third of the broccoli plant, unnecessary preheading sprays to control *P. rapae* larvae can be avoided. However, the presence of large *P. rapae* larvae in broccoli plants at the onset of heading would require immediate control, because these larvae would move into the head and reduce broccoli head quality.

These data also show that coverage of the appropriate part of the broccoli plant by an insecticide is important, especially when applying microbial insecticides such as *B. thuringiensis*. Because *B. thuringiensis* endotoxin needs to be ingested in order to exert its toxic effect, the
knowledge of areas of the broccoli plant which harbor a susceptible pest life stage can be important. For large P. rapae larvae, good coverage of the upper leaves can help ensure that the endotoxin is in the right area for maximum effect. Directing sprays towards the middle and lower portions of the plant could lead to more effective control of P. xylostella larvae by B. thuringiensis, because nearly eighty percent of P. xylostella larvae occur there.

In conclusion, specific life stages of P. rapae and P. xylostella exhibit a vertical distribution on broccoli plants in the field. Significantly more P. xylostella larvae (all instars) and P. rapae small larvae were found in the middle leaves of the broccoli plant than the upper or lower leaves. The upper leaves of the broccoli plant, are the preferred areas for P. rapae oviposition and feeding of large larvae. The numbers of natural enemies for P. rapae were too low during this study to draw any conclusions. No difference in the vertical distribution of P. xylostella pupae or D. insulare cocoons were found among the upper, middle and lower leaves of the plant. The distribution of life stages of these two pests can assist in predicting when and where broccoli plants may be infested and prevent unnecessary use of pesticides.
Chapter 4

Cold Storage of *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) Prepupae in Host Chrysalid
Introduction

*Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae) is a gregarious pupal endoparasite of the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae). It was accidentally introduced into the United States in the late 1800’s (Oatman 1966). Since 1981, studies conducted on cabbage in southwestern Virginia have found seasonal parasitization of *P. rapae* chrysalids by *P. puparum* to consistently average over 50% (Chamberlin & Kok 1986, Lasota & Kok 1986a). This high rate of parasitization makes the use of *P. puparum* promising in augmentative releases for early season control of the imported cabbageworm.

In order to produce large numbers of *P. puparum* for augmentative field releases, manipulation of the parasite in the laboratory may be necessary. Lasota & Kok (1986d) investigated refrigeration of *P. puparum* adults as a method of maintaining and manipulating laboratory colonies. They found that survival of adult *P. puparum* caged in groups and supplied with a 10% sugar solution exceeded 80% after 30 days at both 15°C and 23°C. In order to manipulate large numbers of *P. puparum*, prolonged storage without loss of reproductive viability is necessary.

The objectives of this study were to develop a method for long term storage of *P. puparum* and to determine the maximum storage time that would produce viable *P. puparum* adults.
Materials and Methods

Colonies of *P. puparum* (obtained from field collected hosts) and *P. rapae* (obtained from Paula Peters of the USDA-Biological Control of Insects Research Laboratory in Columbia, MO) were maintained at the Virginia Polytechnic Institute and State University Entomology Department Insectary. Forty 0-24 hour old *P. rapae* chrysalids were placed in a covered petri dish with 10 mated week-old *P. puparum* females. This was replicated five times. *P. puparum* adults were given a 10% sucrose solution which was lightly misted on the lid of the petri dish before parasite addition. After 48 h, the *P. puparum* females were removed, and the parasitized *P. rapae* chrysalids were held in an environmental chamber at 25°C, 16L:8D until parasite prepupae were observed through the translucent chrysalid exoskeleton. The chrysalids containing *P. puparum* prepupae were then stored in an environmental chamber at 10°C and 80% r.h. in total darkness.

Ten randomly selected chrysalids were removed from cold storage at specific monthly intervals and individually placed in a 100 ml cylindrical plexiglass container having an organdy cloth end to facilitate air circulation. The plexiglass cylinders were held at 30°C, 16L:8D and 50% r.h. and monitored daily for parasite emergence. Upon emergence, the number of *P. puparum* adults and sex ratios per chrysalid were recorded. Results obtained from the various durations of 10°C storage treatments were compared to a control which
was not refrigerated, but was maintained at \(25^\circ\text{C}\). Analysis of variance and mean separation tests were used to determine differences in the total number of wasps, and on numbers of female and male wasps emerged at each treatment interval.

To test for parasite viability after cold storage, all \(P.\ puparum\) adults from a single storage period were placed in a 40 cm\(^3\) plexiglass cage. A 10% sucrose solution was lightly misted on the walls of the cage daily. Parasites were allowed to feed and mate for 48 h and were then offered 0-24 h old \(P.\ rapae\) chrysalids to parasitize. Parasitized chrysalids were left in the cage and observed daily for emergence of \(P.\ puparum\) adults.

**Results and Discussion**

\(P.\ puparum\) adults emerged after storage periods of up to 15 months at \(10^\circ\text{C}\) (Table 8), but there was no emergence of adults from the 17 month treatment. Significantly more parasite adults emerged in the control and the four month treatment compared to the other treatment intervals. Differences in numbers of \(P.\ puparum\) adults emerging during the first four months were most likely due to chance selection of parasitized \(P.\ rapae\) chrysalid samples containing large numbers of \(P.\ puparum\) prepupae. More females emerged in the control and at the four month treatment interval when compared to the other treatment times, but there were no differences in the number of males.
Table 8. Mean *P. puparum* adults emerging per chrysalid after storage at 10°C.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>13.7*</td>
<td>5.5</td>
<td>2.2</td>
<td>11.3</td>
<td>4.0</td>
<td>1.5</td>
<td>3.9</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td>(SE)</td>
<td>(2.9)</td>
<td>(1.0)</td>
<td>(0.9)</td>
<td>(2.7)</td>
<td>(2.1)</td>
<td>(0.8)</td>
<td>(2.0)</td>
<td>(0.6)</td>
<td>(0.6)</td>
<td>(0.5)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Male</td>
<td>21.0</td>
<td>15.8</td>
<td>16.1</td>
<td>23.9</td>
<td>5.9</td>
<td>8.6</td>
<td>1.9</td>
<td>2.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>(SE)</td>
<td>(3.8)</td>
<td>(2.9)</td>
<td>(4.9)</td>
<td>(4.3)</td>
<td>(2.2)</td>
<td>(4.5)</td>
<td>(1.1)</td>
<td>(1.6)</td>
<td>(0.0)</td>
<td>(0.4)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>34.7</td>
<td>21.3</td>
<td>18.3</td>
<td>35.2</td>
<td>9.9</td>
<td>10.1</td>
<td>5.8</td>
<td>2.9</td>
<td>0.6</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>(SE)</td>
<td>(4.0)</td>
<td>(3.4)</td>
<td>(4.8)</td>
<td>(5.5)</td>
<td>(3.5)</td>
<td>(4.7)</td>
<td>(2.2)</td>
<td>(1.9)</td>
<td>(0.6)</td>
<td>(0.8)</td>
<td>(0.0)</td>
</tr>
</tbody>
</table>

*Means having the same letter within a row are not different, (P > 0.05, Duncan's Multiple Range Test 1955). For each date, n = 10 *P. rapae* chrysalids.
emerging between treatment intervals during the first four months of storage.

There was a significant decrease in the number of P. puparum adults beginning with the six month treatment. Part of the difference in mortality at the longer storage treatments might be attributed to lack of oxygen in the petri dishes, which were kept closed except for the removal of P. rapae chrysalids during the study. Ventilation of the chrysalids during storage may improve survival of the P. puparum prepupae in the host chrysalid.

Adult P. puparum females emerging from parasitized hosts at each treatment interval were viable, as P. puparum colonies could be started from adults at each storage interval. Only female P. puparum emerged from chrysalids at the fourteen month storage treatment; the arrhenotokous females were fertilized by their male progeny to produce a viable colony. Lasota & Kok's (1986) previous findings that refrigeration did not inhibit viability of adult P. puparum females kept at 10, 15 and 23°C for 30 days can be extended to refrigeration of P. puparum prepupae in the host for periods of up to 15 months without loss of reproductive viability.

These data suggest that P. puparum exhibits a facultative dormancy induced by temperatures of 10°C or less. In southwestern Virginia, P. puparum may be subjected to low temperatures for periods of up to six months, and nutrient reserves in P. puparum prepupae may be depleted.
after this time. At the onset of cold temperatures in the field, parasitized *P. rapae* chrysalids which contain *P. puparum* prepupae and pupae should successfully overwinter. Parasitized chrysalids which contain *P. puparum* larvae that have not yet reached the prepupal or pupal stage may continue to develop during warmer periods above 10°C. The *P. puparum* larvae in the host chrysalid may also successfully overwinter if the host does not die early due to larval feeding by *P. puparum* or dessication of the *P. rapae* chrysalid.

In conclusion, parasitized *P. rapae* chrysalids containing *P. puparum* prepupae can be successfully stored at 10°C and 80% r.h. for periods of up to four months with no significant mortality as compared to a control. Viable colonies of *P. puparum* can be obtained from chrysalids which have been in cold storage for up to fifteen months. Thus, *P. rapae* chrysalids can be sequentially parasitized and refrigerated to stockpile large numbers of *P. puparum* for laboratory studies or augmentative field releases.
Chapter 5

Seasonal Hyperparasitism of *Cotesia glomerata* (L.) and *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) in Southwestern Virginia
**Introduction**

One of the earliest successful introductions of a beneficial insect into the U.S.A. was the establishment in 1883 of the braconid *Cotesia* (formerly *Apanteles*) *glomerata* (L.) (Mason 1981), a gregarious larval endoparasite of the imported cabbageworm, *Pieris rapae* (L.) (Riley 1893). However, Parker (1970) found *C. glomerata* incapable of suppressing *P. rapae* populations in cole crops before economically important injury occurred.

Since the mid-1960’s, biological control workers have attempted to establish another Falearctic braconid, *Cotesia rubecula* (Marshall), a solitary larval parasite of the imported cabbageworm, into the United States. The first North American record of *C. rubecula* came from British Columbia in 1963 (Wilkinson 1966). How *C. rubecula* became established there is unknown. Attempts to introduce the British Columbia biotype into Missouri were unsuccessful (Parker & Pinnell 1972, Nealis 1985).

*C. rubecula* possesses biological attributes which suggest that it may have greater potential in controlling *P. rapae* than *C. glomerata* (Puttler et al. 1970). Parker et al. (1971), and Laing & Corrigan (1987) found *C. rubecula* to dominate when both *C. glomerata* and *C. rubecula* attack the same larvae. In areas where *C. rubecula* was released, *C. glomerata* failed to become an important parasite of *P. rapae* (Parker et al. 1971, Parker & Pinnell 1972).
Both *C. glomerata* and *C. rubecula* are attacked by several hyperparasites (Richards 1941, Parker 1970, Nealis 1983). Hyperparasites have long been recognized as an important facet in the effectiveness of biological control programs (Muesebeck & Dohanian 1927, Rosen 1981). Blunck (1957) stated that hyperparasites in Europe are frequent and diminish the useful effect of *C. glomerata*. Parker et al. (1971) found total hyperparasitization of *C. rubecula* in Missouri varied from 36.6% - 72.4%. In British Columbia, Nealis (1983) reported 39.3% of *C. rubecula* cocoons were hyperparasitized.

This study, conducted in two phases, documents the effect of hyperparasitism on the early stages of introduction and colonization of *C. rubecula* in southwestern Virginia. The first phase consisted of the collection and identification of the hyperparasites attacking *C. glomerata* (1986–1987) and *C. rubecula* (1987). The second phase (1988) quantified the seasonal hyperparasitization of *C. glomerata* and *C. rubecula*. Our objective was to provide a within season description of hyperparasite dynamics and its effect on interspecific competition between a naturalized parasite, *C. glomerata*, and a newly introduced parasite, *C. rubecula*.
Materials and Methods

Cotesia glomerata

An 18 by 25 m plot of broccoli (Packman variety) was planted at the VPI & SU Horticulture Farm during mid-August of 1986, and mid-April and mid-August of 1987. For 1988, a similar sized plot of Packman broccoli was planted in mid-April and mid-August at the VPI & SU Whitethorne farm. Both Whitethorne plantings were also used for the C. rubecula study. Rows were spaced 1 m apart, with plants spaced 0.3 m apart in each row. Two hundred plants were sampled twice weekly in 1986 and 1987, and weekly in 1988 for the occurrence of all life stages of P. rapae and C. glomerata. Cocoon masses of C. glomerata were collected during sampling and taken to the laboratory. Cocoons in each mass were individually separated and isolated in size 00 gelatin capsules. All isolated cocoons from a single mass were placed in a 236.5 ml (8 oz.) wax sundae cup and held in an environmental chamber under long day conditions 16L:8D, 25°C and 50% R.H. until emergence of primary or secondary parasites. Data recorded were: date of collection of the cocoon mass; number and identity of the parasite or hyperparasite(s) emerging from each cocoon; and sex ratios of emerged parasites and hyperparasites.
**Cotesia rubecula**

We obtained a Yugoslavian strain of *C. rubecula* from Ben Puttler (USDA-BCIRL, Columbia, Mo.) in May 1987 to start a colony at the VPI & SU insectary. Broccoli plots (Packman variety) were planted in mid-April at two locations near the VPI & SU campus, Prices Fork and Whitethorne. The Prices Fork plot measured 15 by 15 m; the Whitethorne plot measured 18 by 25 m. Rows were spaced 1 m apart, with plants spaced 0.3 m apart in each row. During 1987, 198 *C. rubecula* adults (1:1 sex ratio) were released at the Prices Fork site, and 500 adults (1:1 sex ratio) were released at Whitethorne.

To determine the presence of hyperparasites, two collections of *C. rubecula* cocoons were taken from the field during July 1987. Each cocoon was placed into a size 00 gelatin capsule, and all isolated cocoons from a single collection date were placed in a 236.5 ml wax sundae cup and maintained in an environmental chamber at 16L:8D, 25°C and 50% R.H. until emergence of the parasite or hyperparasite(s).

The broccoli plots at Prices Fork and Whitethorne were replanted in mid-April of 1988 and sampled for the presence of *C. rubecula*. Collections of a minimum of 10 *C. rubecula* cocoons were made on a weekly basis from July until early August 1988 at Whitethorne and taken to the laboratory for encapsulation and observation. In mid-August, broccoli plots measuring 6 by 6 m were planted at 0.5, 0.8, and 1.2
km in a northeasterly direction from the original release site at Whitethorne. These plots were used to monitor dispersal of *C. rubecula* and were sampled weekly for cocoons of *C. glomerata* and *C. rubecula*. Cocoon masses of *C. glomerata* found at these plots were taken to the laboratory for observation.

Differences in the level of hyperparasitization between *C. glomerata* (1986-1988) and *C. rubecula* (1987-1988) were compared using summary statistics involving the Z test (Lentner 1984).

All hyperparasite species recovered from *C. glomerata* and *C. rubecula* were sent to the USDA-ARS Taxonomic Services Unit, Systematic Entomology Laboratory, Plant Sciences Institute, Beltsville, Md., for identification.

**Results and Discussion**

**Cotesia glomerata**

The following hyperparasites were recovered from 1986-1988: *Catolaccus aeneoviridis* (Girault) (Hymenoptera: Pteromalidae), *Spilochalcis torvina* (Cresson) (Hymenoptera: Chalcididae), and *Tetrastichus galactopus* (Ratzeburg) (Hymenoptera: Eulophidae). Figure 18 shows the relationships between *C. glomerata*, *C. rubecula*, and their respective hyperparasites during the course of this study. Table 9 summarizes the level of hyperparasitization of *C. glomerata*
HYPERPARASITES

T. galactopus

S. torvina

C. aeneoviridis

I. lycaenae

PARASITES

C. glomerata

C. rubecula

HOST

P. rapae

Fig. 18. Relationships between P. rapae, its larval parasites C. glomerata and C. rubecula, and their respective hyperparasites.
Table 9. Mean percentage of hyperparasites recovered from *C. glomerata* cocoons across all plots from 1986-1988. The remaining percentages for each year were cocoons with no insect emergence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean(SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1986 (n = 412)</strong></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>50.7(2.5)</td>
</tr>
<tr>
<td><em>T. galactopus</em></td>
<td>3.4(0.8)</td>
</tr>
<tr>
<td><strong>1987 (n = 189)</strong></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>0.0</td>
</tr>
<tr>
<td><em>C. aeneoviridis</em></td>
<td>3.2(1.2)</td>
</tr>
<tr>
<td><em>S. tcrvina</em></td>
<td>1.1(0.7)</td>
</tr>
<tr>
<td><em>T. galactopus</em></td>
<td>61.4(3.5)</td>
</tr>
<tr>
<td><strong>1988 (n = 145)</strong></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>60.2(1.2)</td>
</tr>
<tr>
<td><em>C. aeneoviridis</em></td>
<td>1.2(0.3)</td>
</tr>
<tr>
<td><em>S. tcrvina</em></td>
<td>0.9(0.3)</td>
</tr>
</tbody>
</table>
from 1986-1988; seasonal hyperparasite activity for each of the three years of this study is shown in Fig. 19.

*C. aeneoviridis* has been commonly recorded as both a primary and secondary solitary parasite on a wide range of host insect pupae (Muesebeck et al. 1951, Peck 1963, Puttler 1966). The earliest record of *C. aeneoviridis* in Virginia comes from Gould & Geissler (1940), who recorded it as a parasite of the pistol case bearer, *Coleophora malivorella* Riley. Hill & Hough (1957) reported *C. aeneoviridis* as a secondary parasite attacking *Apanteles ornigis* Weed in Virginia.

*S. torvina* is a solitary pupal endoparasite and has also been recorded as both a parasite and hyperparasite from a wide range of hosts (Arthur 1958, Burks 1940). Chittenden (1920) and Hough (1927) reported *Smicra* (=*Spilochalcis*) *torvina* in Virginia as a primary parasite attacking the red-banded leaf roller, *Argyrotaenia velutinana* (Walker). *S. torvina* (as *S. side*) has been recorded in crucifer plantings as a primary parasite of *Ceutorhynchus assimilis* (Payk.) (Carlson et al. 1951, McLeod 1953), *Plutella xylostella* (L.) (Harding 1976a, McNeil & Rabb 1973), *Trichoplusia ni* (Hubner) and *Pseudoplusia includens* (Walker) (Harding 1976b), and as a hyperparasite of *Diadegma insulare* (=*insularis*) (Cresson) (Marsh 1917, McNeil & Rabb 1973), and *Cotesia glomerata* (L.) (McNeil & Rabb 1973, Parker 1970).
Fig. 19. Hyperparasitization of *C. glomerata* cocoon samples from 1986–1988. Remaining percentages for each date were cocoons that failed to produce insects.
S. torvina is currently listed as a synonym of S. side (Walker), but has been given species status by Couch (1984).

T. galactopus is a gregarious endoparasite of braconid larvae, ovipositing directly into the parasite larvae through the body wall of the primary host. It is thought to have entered North America with C. glomerata in the 1880's (Nealis 1983). Nealis (1983) reviewed the biology and life history of T. galactopus.

The within season level of hyperparasitization of C. glomerata from 1986-1988 was variable (Fig. 19). Except for 16 October, hyperparasitization was low in 1986. Due to the absence of C. glomerata at the VPI & SU Horticulture Farm in 1987, a single collection of seven C. glomerata cocoon masses was made on 30 July at the Whitethorne farm; these were heavily hyperparasitized by T. galactopus (61.4%). In 1988, samples taken on 13 July, 21 July and 25 October had high proportions of hyperparasitized hosts. We do not know why the proportion of unemerged cocoons was high on some dates; it could be partly due to handling of the individual cocoons when separating each from the mass. However, Parker (1970) found that C. glomerata emerged from only 8% of marked cocoons in the field.

Cotesia rubecula

P. rapae larvae parasitized by C. rubecula were observed throughout the 1987 growing season at both the Prices Fork and Whitethorne locations. New cocoons of C.
rubecula were found as late as 29 October 1987 at the Whitethorne site.

On 29 June 1988, cocoons of C. rubecula were found in the broccoli plot at Whitethorne, indicating that the parasite had successfully overwintered. No recovery of C. rubecula occurred at the Prices Fork site. In the plots planted to monitor dispersal at Whitethorne, a C. rubecula cocoon was found in the 0.8 km plot on 28 September 1988 and another was found on 2 November 1988, indicating that C. rubecula had dispersed from the original release site.

The following hyperparasites were recovered from C. rubecula during 1987 and 1988: Isdromas lycaenae (Howard) (Hymenoptera: Ichneumonidae), S. torvina, and T. galactopus (Table 10, Fig. 20).

I. lycaenae is a solitary pupal hyperparasite of Cotesia (=Apanteles) spp., Meteorus spp., Campoletis spp., and Hyposoter spp. (Muesebeck et al. 1951). We found it attacking C. rubecula but not C. glomerata (Fig. 18).

In 1988, we recovered S. torvina from the pupae of C. glomerata, C. rubecula, P. xylostella, D. insulare, and an unidentified brown cocoon, possibly another parasite of the diamondback moth, Microplitis plutellae (Cress.).

The diversity and number of hyperparasite species can vary greatly from place to place and from year to year (Sullivan 1987). For example, in 1986 and 1987, T.
Table 10. Mean percentage of hyperparasites recovered from *C. rubecula* cocoons across all plots during 1987 and 1988. The remaining percentages for each year were cocoons with no insect emergence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean(SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1987 (n = 22)</strong></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>40.9(10.7)</td>
</tr>
<tr>
<td><em>S. torvina</em></td>
<td>9.1(6.2)</td>
</tr>
<tr>
<td><em>T. galactopus</em></td>
<td>22.7(9.1)</td>
</tr>
<tr>
<td><strong>1988 (n = 52)</strong></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>33.3(6.7)</td>
</tr>
<tr>
<td><em>I. lycaenae</em></td>
<td>1.9(1.9)</td>
</tr>
<tr>
<td><em>S. torvina</em></td>
<td>39.2(6.9)</td>
</tr>
</tbody>
</table>
Fig. 20. Hyperparasitization of *C. rubecula* cocoon samples for 1987–1988.
Remaining percentages for each date were cocoons that failed to produce insects.
**galactopus** was recovered as the dominant hyperparasite of both *C. glomerata* and *C. rubecula*, but it was not recovered in 1988.

The hyperparasitization levels of *C. rubecula* increased during the late season in both 1987 and 1988 (Fig. 20). Eighty percent of the cocoons collected on 1 July 1987 produced *C. rubecula* adults. However, only 8% of the cocoons collected on 30 July 1987 produced *C. rubecula* adults; 50% of the cocoons were hyperparasitized and the remaining 42% of the cocoons did not produce any insects. In 1988, the same pattern of increased late season hyperparasitization occurred. Only hyperparasites emerged from *C. rubecula* cocoons collected on 5 August 1988. After this date in 1988, *C. rubecula* cocoon levels in the field were too low to detect with our sampling techniques.

Based on 1988 seasonal data, the population dynamics of *C. glomerata* and *C. rubecula* appear to be dictated by the hyperparasites attacking *C. rubecula*, especially *S. torvina* (Fig. 21). In the early season, hyperparasite activity by *S. torvina* was low, and *C. rubecula* outcompeted *C. glomerata* for hosts. This explains *C. rubecula*'s dominance from late June until early August. On 5 August 1988, hyperparasitization of *C. rubecula* peaked at 100% of cocoons that had insect emergence (83.3% by *S. torvina* and 16.7% by *I. lycaenae*). This was followed by a dramatic decline in the number of *C. rubecula* cocoons in the field. In the absence
Fig. 21. Population dynamics of Cotesia species and hyperparasitization of C. rubecula in 1988.
of *C. rubecula*, *C. glomerata* became the dominant parasite of *P. rapae* larvae during late September and early October. During the late season (mid-October to early November) *C. rubecula* populations recovered slightly, perhaps due to lessened hyperparasite activity, as observed by Parker et al. (1971) in Missouri. These results confirm the competitiveness of *C. rubecula* versus *C. glomerata* as indicated by Puttler et al. (1970), Parker et al. (1971), Parker & Pinnell (1972) and Laing & Corrigan (1987). Thus, with the introduction of *C. rubecula*, the populations of *C. glomerata* can be expected to decline, unless hyperparasitism keeps the former in check.

There was a significant difference between hyperparasitization of *C. glomerata* during 1986-1988 (8.1%) and *C. rubecula* during 1987-1988 (37.9%) (Table 11). We believe that hyperparasitism of *C. rubecula*, which was nearly five times higher than that of *C. glomerata*, had a profound effect upon the population dynamics and interspecific competition between these two parasite species for their mutual host, *P. rapae*.

We were unable to recover *C. rubecula* at the Whitethorne release site during the 1989 growing season. All indications appear that the absence of *C. rubecula* in our field plots was due to the action of hyperparasites, particularly the pupal hyperparasite *G. torvina*, which

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Female</th>
<th>Male</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. glomerata Combined Total for 1986-1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>1083</td>
<td>663</td>
<td>420</td>
<td>52.7</td>
</tr>
<tr>
<td>C. aeneoviridis</td>
<td>23</td>
<td>6</td>
<td>17</td>
<td>1.1</td>
</tr>
<tr>
<td>S. torvina</td>
<td>17</td>
<td>7</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>T. galactopus</td>
<td>128</td>
<td>25</td>
<td>144</td>
<td>6.2</td>
</tr>
<tr>
<td>No emergence</td>
<td>803</td>
<td>-</td>
<td>-</td>
<td>39.2</td>
</tr>
<tr>
<td><strong>Hyperparasitization from 72 Cocoon masses (2054 cocoons) = 8.1%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. rubecula Combined Total for 1987-1988</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>26</td>
<td>10</td>
<td>16</td>
<td>35.1</td>
</tr>
<tr>
<td>L. lycaenae</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1.4</td>
</tr>
<tr>
<td>S. torvina</td>
<td>22</td>
<td>15</td>
<td>7</td>
<td>29.7</td>
</tr>
<tr>
<td>T. galactopus</td>
<td>5</td>
<td>31</td>
<td>5</td>
<td>6.8</td>
</tr>
<tr>
<td>No emergence</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>27.0</td>
</tr>
<tr>
<td><strong>Total hyperparasitization from 74 Cocoons = 37.9%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary Statistic: Z Test

Square root of: (.08)(.92)/2054 + (.38)(.62)/74 = 0.05674

\[ Z = \frac{p_1 - p_2}{0.05674} = 0.38 - 0.08/0.05674 = 5.29^a \]

\(^a\)Percentage of hyperparasitized C. glomerata cocoons significantly different from percentage of hyperparasitized C. rubecula cocoons using the Z test (\(\alpha = 0.05\), Lentner 1984).
overwhelmed the *C. rubecula* population within a year of its initial release.

Nealis (1983) concluded that hyperparasitism was not a primary factor limiting the establishment of *C. rubecula* in North America. However, this study indicates that hyperparasitism can be a primary limiting factor in the establishment and proliferation of *C. rubecula* in southwestern Virginia and perhaps in other areas of North America where *S. toryina* is present.

In conclusion, *C. rubecula* was attacked by indigenous hyperparasites in its new habitat soon after introduction. In spite of hyperparasitism, *C. rubecula* was recovered in three consecutive planting seasons between 1987 and 1988. As *C. rubecula* was initially released into a monoculture of broccoli, it may have been exposed to an unusually high amount of hyperparasitization due to the simplified ecosystem into which it was introduced. Our recovery of *C. rubecula* during late June of 1988 also suggests that it completed an earlier generation outside our plot, presumably in nearby stands of wild cruciferae. Dispersal was also confirmed by the recovery of 2 *C. rubecula* cocoons at the 0.8 km broccoli plot.

We hypothesize that hyperparasite activity against *C. rubecula* in pastoral areas containing wild cruciferae may be lower than that found in the agricultural ecosystem, due to the increased diversity present in them as compared to a
monoculture of cole crops. An alternate release strategy for biocontrol agents proven sensitive to hyperparasitism would be to place them into the most stable ecosystem possible. Basically, this is an extension of Russell's (1989) natural enemies hypothesis. In our case, it would mean releasing C. rubecula into pastoral areas containing wild cruciferae which are also hosts for P. rapae. Corrigan's (1982) recovery of C. rubecula on four small cabbage plants in a back yard garden in Ottawa, Ontario, a decade after its release and approximately 30 km from the original release site lends credence to this view, since no recovery of C. rubecula in the agricultural ecosystem was reported during that time. Too frequently in biological control, there is inadequate documentation on the reasons for the success or failure of a biotic agent to establish. If we are to improve the success rate of natural enemy establishments and our understanding of ecology, the reasons for failure as well as success should be emphasized.
Chapter Six

Susceptibility of Pieris rapae (L.) Larvae Parasitized by the Braconid Cotesia rubecula (Marshall) to Sublethal Dosages of Bacillus thuringiensis Berliner subsp. kurstaki.
Introduction

The microbial pesticide *Bacillus thuringiensis* Berliner is being used with increasing frequency in the agricultural ecosystem. The *B. thuringiensis* delta endotoxin needs to be ingested by target pest larvae in order to cause mortality; thus, adequate foliar coverage of the pesticide has been a major concern for growers and pest management specialists. Plants sprayed with *B. thuringiensis* have varying amounts of endotoxin on leaf surfaces, and the amount of toxin decreases as exposure to ambient field conditions increases (Leong et al. 1980).

Studies conducted at Virginia Polytechnic Institute & State University from 1981-1984 have shown the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae) to be the predominant pest of crucifers in southwestern Virginia (Chamberlin & Kok 1986, Lasota & Kok 1989). These studies have also shown *Cotesia glomerata* (L.) (Hymenoptera: Braconidae), a gregarious larval endoparasite of *P. rapae*, to average 25% seasonal parasitization. Although *C. glomerata* was introduced into the United States in 1883 to reduce imported cabbageworm populations, and is one of its most common parasites, it does not suppress populations of *P. rapae* below damaging levels (Parker 1970).

Since *P. rapae* is native to Europe, its complement of specific natural enemies may be lacking in North America.
(Blunck 1957, Puttler et al. 1970). In the Palearctic region, the most common parasite of *P. rapae* is *Cotesia rubecula* (Marshall) (Richards 1941, Blunck 1957). *C. rubecula* is a solitary endoparasite of *P. rapae* larvae. In the United States, the major larval parasite of the imported cabbageworm is *C. glomerata* (Blunck 1957).

*C. rubecula* has four biological attributes which suggest that it may have a greater potential in controlling *P. rapae* than *C. glomerata*. First, *C. rubecula* is almost host specific to *P. rapae*, while in the Palearctic region, *C. glomerata* is primarily a parasite of *Pieris brassicae* (L.) (Blunck 1957, Puttler et al. 1970). Second, eggs of *C. rubecula* are not encapsulated when laid in first- through third-instar *P. rapae* larvae, indicating a more adapted host-parasite relationship than *C. glomerata*, whose eggs are encapsulated in mid-second and third instar larvae (Puttler et al. 1970). Third, *C. rubecula* was found to be superior to *C. glomerata* when both parasites attack the same larva (Parker et al. 1971, Laing & Corrigan 1987). Fourth, Rahman (1970) and Parker & Pinnell (1973) found that *P. rapae* larvae parasitized by *C. rubecula* consume approximately 1/8th the leaf area in comparison to unparasitized *P. rapae* larvae.

The potential for integrating natural enemies with microbial pesticides holds promise, since microbial
pesticides such as B. thuringiensis are selective in their toxicity. The effect of B. thuringiensis on parasitized larvae merits investigation, since little research has been done in this area. The objective of this study was to investigate dose-response differences of parasitized versus unparasitized 4th instar P. rapae in the laboratory and to determine the effect of specific dosages of bt and to determine the effect of specific dosages of B. thuringiensis on the ability of C. rubecula to successfully pupate.

Materials and Methods

Colonies of P. rapae and C. rubecula were maintained at the Virginia Polytechnic Institute & State University Insectary facilities. For bioassays, parasitized P. rapae larvae were obtained using a method described by Parker & Pinnell (1970). The formulation of B. thuringiensis used for the laboratory tests was ABG-6167 (B. thuringiensis subsp. kurstaki), an experimental aqueous suspension from Abbott Laboratories. ABG-6167 contained 16.9 Billion International Units (BIU) of potency per liter.

Initial bioassays of unparasitized P. rapae fourth instar larvae (four replicates of ten larvae at each dose) were conducted at serial dilutions of 850, 85, 8.5, 0.85, 0.085, and 0.0085 international units of B. thuringiensis per ml. Each dilution was surface incorporated on a wheat
germ diet in an 236 ml (8 oz.) wax cup. Ten newly molted fourth instar *P. rapae* larvae were placed in the cup. The cup was placed in an environmental chamber at 30°C, 16L:8D and 50% r.h. Mortality of the larvae were recorded on a daily basis for seven days. From the initial test, dosages of *B. thuringiensis* of 850, 85.0, and 8.5 international units were selected for feeding tests on unparasitized and parasitized larvae. Thirty replicates of 10 larvae per cup were used for each dosage of *B. thuringiensis*. Cups were held at 30°C, 16L:8D, and 50% r.h. Mortality was recorded every 24 h.

Probit analysis (SAS Institute 1985) was used to calculate LC50's for unparasitized and parasitized larvae. Linear regression was used to determine the response slopes for the two groups of larvae for each of the seven days. The daily LC50 response ratios for parasitized versus unparasitized larvae were calculated for comparison. The number of parasitized larvae which had successful emergence of *C. rubecula* was recorded daily and the percent calculated.

Results and Discussion

*P. rapae* fourth instars parasitized by *C. rubecula* responded differently to *B. thuringiensis* when compared to unparasitized larvae at the same levels of exposure (Table
After day two, the LC$_{50}$'s of parasitized *P. rapae* fourth instars were approximately thirty times higher than unparasitized larvae. By day four, the LC$_{50}$ response of parasitized larvae was 180 times greater than unparasitized larvae, and by day seven, it rose to 737 times greater than unparasitized larvae. The differential mortalities between unparasitized and parasitized larvae on days 5, 6, and 7 were due mainly to the increased mortality of unparasitized larvae affected by *B. thuringiensis*, since *C. rubecula* eonymphs exited from the majority of parasitized fourth instars by day four (Figure 22).

Twenty-five percent of parasitized fourth instars exposed to a concentration of 850 I.U./ml successfully pupated, compared to 76% successful pupation at 85 I.U./ml and 69% at 8.5 I.U./ml. The lower percentage of successful pupation at 8.5 I.U./ml compared to 85 I.U./ml may be due to biological variation among the larvae.

These results show that fourth instar *P. rapae* larvae parasitized by *C. rubecula* are less susceptible to low dosages of *B. thuringiensis* than unparasitized larvae, which indirectly support Rahman's (1970) and Parker & Pinnell's (1973) findings that parasitized larvae consume less food than do unparasitized larvae.

The differential susceptibility between parasitized and unparasitized larvae could be used as a possible control
Table 12. Response of fourth instar *P. rapae* parasitized by *C. rubecula* compared to unparasitized fourth instars. Both were fed on a wheat germ diet which was surface-treated with *B. thuringiensis*. Mortality was recorded daily for 7 d.

<table>
<thead>
<tr>
<th>Day</th>
<th>Slope ± SE</th>
<th>LC$_{50}$</th>
<th>Slope ± SE</th>
<th>LC$_{50}$</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unparasitized$^a$</td>
<td></td>
<td>Paratized$^a$</td>
</tr>
<tr>
<td>1</td>
<td>0.190 ± 0.065</td>
<td>42952.0</td>
<td>0.157 ± 0.070</td>
<td>10678.9</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.096 ± 0.045</td>
<td>376.0</td>
<td>0.276 ± 0.049</td>
<td>583.8</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>0.080 ± 0.046</td>
<td>12.2</td>
<td>0.248 ± 0.049</td>
<td>380.2</td>
<td>31.2</td>
</tr>
<tr>
<td>4</td>
<td>0.101 ± 0.046</td>
<td>1.8</td>
<td>0.227 ± 0.049</td>
<td>319.5</td>
<td>182.6</td>
</tr>
<tr>
<td>5</td>
<td>0.118 ± 0.056</td>
<td>1.5</td>
<td>0.226 ± 0.047</td>
<td>295.3</td>
<td>196.9</td>
</tr>
<tr>
<td>6</td>
<td>0.085 ± 0.073</td>
<td>0.9</td>
<td>0.223 ± 0.047</td>
<td>273.7</td>
<td>300.8</td>
</tr>
<tr>
<td>7</td>
<td>0.099 ± 0.094</td>
<td>0.4</td>
<td>0.224 ± 0.046</td>
<td>258.0</td>
<td>737.2</td>
</tr>
</tbody>
</table>

$^a$ n = 900 larvae

$^b$ LC$_{50}$ expressed in International Units of *B. thuringiensis* delta endotoxin potency per ml.

$^c$ Response Ratio - LC$_{50}$ parasitized larvae/LC$_{50}$ unparasitized larvae.
Fig. 22. Percentage pupation of *C. rubecula* in *B. thuringiensis*-intoxicated 4th instar *P. rapae* larvae. n = 300 at each dosage.
tactic in the field. *C. rubecula* may have the potential to be integrated into an IPM program utilizing timed applications of *B. thuringiensis* sprays. Since *C. rubecula* exits from 4th instar larvae, sprays could be timed to coincide with the appearance of the fifth instar larvae. By this time, most of the *C. rubecula* eonymphs would have emerged from the parasitized fourth instars. This would cause maximum mortality to the *P. rapae* larval population and minimal mortality to the *C. rubecula* population. This management scheme could conserve natural enemies for better control of late season crucifer pests (e.g. *Trichoplusia ni* (Hübner)).
SUMMARY

Three formulations of Bacillus thuringiensis Berliner subsp. kurstaki were compared with the synthetic pyrethroid permethrin (common name Pounce 3.2 EC) for insecticidal efficacy and impact upon parasitism of the imported cabbageworm, Pieris rapae (L.) (Lepidoptera: Pieridae) in four seasons of field broccoli from fall 1986 to spring 1988. The three B. thuringiensis formulations were: ABG-6158, an experimental oil emulsion, now commercially available as Dipel 4L; ABG-6167, an experimental aqueous suspension; and Dipel 2X, a commercially available wettable powder. The results of four seasons of field tests showed that permethrin, ABG-6167, and Dipel 4L were not significantly different in terms of insecticidal efficacy towards P. rapae larvae.

Natural enemy activity by the larval parasite Cotesia glomerata (L.) (Hymenoptera: Braconidae) and the pupal parasite Pteromalus puparum (L.) (Hymenoptera: Pteromalidae) continued after spraying in the B. thuringiensis treatments, but was too low to be significantly different from the permethrin treated plots. However, the percentage of P. rapae chrysalids parasitized by P. puparum was extremely high (100% of P. rapae chrysalids recovered). P. puparum has a high host finding capacity even at low levels of its host, and has potential to be integrated into a pest management program incorporating selective microbial insecticides such as B. thuringiensis.
A method was devised for long term cold storage of *P. puparum*. Parasitized *P. rapae* chrysalids containing *P. puparum* prepupae could be stored at 10°C and 80% r.h. for periods of up to four months with little mortality as compared to a control. Viable colonies of *P. puparum* adults could be obtained from chrysalids which had been in cold storage for periods of up to fifteen months. Thus, parasitized *P. rapae* chrysalids could be sequentially parasitized and refrigerated to stockpile large numbers of *P. puparum* for laboratory studies or field releases.

The immature life stages of the imported cabbageworm, the diamondback moth, and their parasites exhibited a vertical distribution on broccoli plants in the field. The leaves of the upper third of the broccoli plant, which are higher in nitrogen, contained significantly more *P. rapae* eggs and large larvae (instars 4-5) than leaves of the middle or lower third of the plant. More *P. rapae* small larvae (instars 1-3) and *P. xylostella* larvae (all instars) were found in the middle third of the broccoli plant than the upper or lower third. No difference by leaf position was found for *P. xylostella* pupae or the diamondback moth larval parasite *Diadegma insulare* (Cresson). Levels of *C. glomerata* and *P. puparum* were low and no meaningful conclusions on vertical distribution of *P. rapae* parasites could be drawn.

Four hyperparasites, two of them attacking both *C. glomerata* and *C. rubecula*, larval parasites of the imported cabbageworm, were found in the field from 1986-1988. The
level of hyperparasitization for the two primary parasites, however, was significantly different. Hyperparasitization averaged only 8.1% for C. glomerata from 1986-1988, but was 37.9% for C. rubecula from 1987-1988.

Hyperparasites recovered from C. glomerata were: Tetrasichus galactopus (Ratzeburg) (Hymenoptera: Eulophidae) (6.2%), Catolaccus aeneoviridis (Girault) (Hymenoptera: Pteromalidae) (1.1%) and Spilochalcis torvina (Cresson) (Hymenoptera: Chalcididae) (0.8%). The former attacked the larval stage; the latter two were pupal hyperparasites. Hyperparasites recovered from C. rubecula were: S. torvina (29.7%), T. galactopus (6.8%) and a pupal hyperparasite, Isdromas lycaenae (Walker) (Hymenoptera: Ichneumonidae) (1.4%).

During the early-to mid-season of 1988, hyperparasite activity was low and C. rubecula outcompeted C. glomerata for hosts; but by mid-season, hyperparasite activity against C. rubecula increased to 100%, causing its populations to crash. C. glomerata, which was less affected by hyperparasite activity and freed from its intrinsically superior competitor, became the dominant parasite of P. rapae. During the late season of 1988, C. rubecula populations recovered slightly. We were unable to recover C. rubecula in our broccoli plots during the 1989 growing season. Hyperparasites, especially S. torvina, appear to be a primary limiting factor in the establishment of C. rubecula in southwestern Virginia, and may also adversely
affect the establishment of *C. rubecula* in other areas of North America where *S. torvina* is present.

In the laboratory, fourth instar *P. rapae* parasitized by *C. rubecula* responded differently to *B. thuringiensis* when compared to unparasitized larvae at the same levels of exposure. After day two, the LC50's of parasitized *P. rapae* larvae were approximately thirty times higher than unparasitized larvae, and by day four, the LC50's of parasitized larvae were 180 times higher than unparasitized larvae. Twenty-five percent of parasitized *P. rapae* larvae exposed to a concentration of *B. thuringiensis* of 850 I.U./ml successfully emerged and pupated, compared to 76% successful pupation at 85 I.U./ml and 69% at 8.5 I.U./ml (n = 300 larvae at each dosage). These results show that fourth instar *P. rapae* larvae parasitized by *C. rubecula* are less susceptible to *B. thuringiensis* than unparasitized larvae, mainly because parasitized larvae consume less food in the fourth instar than do unparasitized larvae.
Literature Cited


Lasota, J. A. and L. T. Kok. 1989. Seasonal abundance of imported cabbageworm (Lepidoptera: Pieridae), cabbage looper (Lepidoptera: Noctuidae), and diamondback moth (Lepidoptera: Plutellidae) on cabbage in southwestern Virginia. J. Econ. Entomol. 82 (3): 811-818.


Tanada, Y. 1953. Susceptibility of the imported cabbage worm to *Bacillus thuringiensis* Berliner. Ha. Ent. Soc. 15: 159-166.


Wollam, J. D. and W. G. Yendol. 1976. Evaluation of *Bacillus thuringiensis* and a parasitoid for suppression of the gypsy moth. J. Econ. Entomol. 69: 113-118.
Appendix I

An Efficient Method For Laboratory Propagation of *Cotesia rubecula* (L.) (Hymenoptera: Braconidae)
Introduction

*Cotesia* (=*Apanteles*) *rubecula* (Hymenoptera: Braconidae), a palearctic larval parasite of the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae) possesses many traits as a biological control agent which make its establishment in the United States desirable (Puttler et al. 1970). The efficient laboratory production of vigorous, disease free individuals of *C. rubecula* for field release would expedite its establishment. Personal experience with Parker & Pinnell's (1970) methods for rearing *C. rubecula* proved unsatisfactory, primarily due to time constraints required for diet preparation, oviposition unit and rearing cage setup times and the occurrence of viral, bacterial (*Serratia* spp.), and fungal (predominantly *Aspergillus* spp.) contaminants in the diet. An alternative method of rearing *P. rapae* parasitized by *C. rubecula* using cruciferous plant material instead of diet is presented.

Materials and Methods

A Yugoslavian strain of *C. rubecula* was obtained in May 1987 from Ben Puttler, a senior research scientist at the ARS-USDA Biological Control of Insects Research Laboratory in Columbia, Mo. A *P. rapae* colony and rearing methods were obtained in August 1986 from Paula Peters, head of the insectary at the aforementioned laboratory.

*P. rapae* Oviposition Unit. A colony of 50 to 100 *P. rapae* adults were maintained in a cage at the Entomology
Insectary, VPI & SU. A 29.6 ml (1 oz.) clear plastic diet cup (4.0 cm in height) was used as an ovipositional unit with the following modifications. The cup was half filled with tap water and a young broccoli leaf (approximately 5 cm in diameter) was placed over the top and secured around the outside with a rubber band. Water in the cup aided in retarding desiccation of the leaf. Excess leaf material greater than 1 cm below the rubber band was trimmed away. A strip of brown paper toweling was wrapped around the cup, leaving 2 cm on either end which was used to hold the toweling to the cup with a paper clip. Toweling height was 2 mm below the top of the cup. The height of the toweling did not impede tarsal contact of the broccoli leaf by the female butterflies (needed as a stimulus for oviposition) but was not low enough for the females to oviposit above it. Toweling was superior to waxed paper or Parafilm for the following reasons: it allowed the base of the eggs to be sterilized; it was easier to manipulate during sterilization; and it was less expensive. The paper toweling and leaf were changed every 24 hours.

Each paper toweling strip with eggs was dated, enclosed in a 236.5 ml (8 oz.) wax sundae cup with lid and refrigerated at 10°C and 80% R. H. Up to fourteen egg strips could be kept per cup. Eggs were allowed to "harden" for 2-4 days in refrigeration before sterilizing. Toweling strips with eggs were sterilized in 10% formaldehyde solution for 15 minutes and rinsed in gently running cold tap water for 1
h. After drying, the toweling with eggs could once again be refrigerated in the cups at 8-10°C and 80% relative humidity for 7-10 days. Normal eclosion time for *P. rapae* eggs at 30°C averaged 3 days.

C. *rubecula* Oviposition Cage. Greenhouse grown broccoli plants 8 to 10 weeks old (Cultivar "Packman" - 8 to 10 leaf stage) were used as the host plant. A piece of sterilized paper toweling with approximately 35 *P. rapae* eggs (2.0 - 2.5 cm²) was cut and placed in the central whorl of the plant with the eggs facing a new leaf. Upon eclosion, first instars immediately migrate to the succulent leaf surface and begin to feed. Four plants with eggs sheets were placed in an oviposition cage 0.5 m (h) by 1.0 m (w) by 0.5 m (d) made of 2.0 cm CDX plywood on the 0.5 m² sides and bottom, with a plexiglas top; the back of the cage was covered with organdy cloth. Each 0.5 m² side had two organdy-covered holes 10 cm in diameter which provided additional ventilation. The cage front was hinged and had two canvas sleeve openings for access. The cage was located under a north-facing window in the anteroom of the insectary, which provided ambient light. Once the majority of larvae (ca. 140 per cage) had eclosed (1-3 days), the egg sheets were removed from the plants.

Six mated 3- to 7-day old *C. rubecula* females were introduced into the cage individually in size 00 gelatin capsules. Each female was released in the center of a plant; oviposition immediately commenced in the majority of cases.
The wasps were fed a 10% sucrose solution which was misted 1-2 times daily on the inside walls and top of the cage with a plant mister. After 48 h the plants were accessed through the canvas sleeves and gently tapped to dislodge any resting females. The parasites exhibited a positive phototactic response to this disturbance which allowed for the rapid removal of plants without parasite escape from the cage. Additional plants with newly eclosed host larvae from a second cage could also be added at this time. Larvae exposed to the parasites for greater than 48 h were often superparasitized.

**Greenhouse Rearing Cage for Parasitized Larvae.** The *P. rapae* larvae exposed to the wasps were transported on the four broccoli plants to an adjoining greenhouse and placed into a 0.5 m³ cage with 8 by 16 mesh window screening on all sides. The cage was elevated from the surface of the greenhouse bench on terra cotta planting pots immersed in aluminum pie pans (20 cm in diameter) filled with water. The pie pans were filled with water daily, preventing the invasion of ants which preyed upon first and second instar larvae. Four additional uncolonized broccoli plants (8-10 leaf stage) were placed in contact with the plants with parasitized larvae. This enabled the larvae to have adequate leaf area upon which to feed and complete development. The cage was placed under a long-day lighting regime (16L:8D).

**C. rubecula Colony Maintenance.** *C. rubecula* cocoons were harvested from the rearing cages and individually placed in
gelatin capsules. The number of cocoons per setup was recorded. Gelatin capsules containing cocoons were placed in 236.5 ml (8 oz.) wax sundae cups and incubated in an environmental chamber at 30°C (± 0.5°C) and 40% relative humidity under continuous light. The gelatin capsules were checked every 24 hours. Upon emergence, the wasps in capsules were placed in a refrigerator for 15 minutes to slow locomotor activity. Individuals were inspected and the sex of each was recorded. A colony cage (0.5 m³) consisting of a 1:1 female/male sex ratio was maintained; excess males were kept in a separate cage and used for sex ratio maintenance or field releases. Each cage had a glass top, organdy cloth back, two canvas sleeve accesses (one on the front of the cage and one on a side) and a hinged front. Both cages were exposed to combined natural and fluorescent light regimes under ambient insectary anteroom temperatures of 27°C (± 3°C). Wasps were given a 10% sucrose solution daily which was misted on the sides of the cage.

Results and Discussion

The time from C. rubecula oviposition in host larvae to emergence of parasite eonymphs from fourth-instar larvae in the greenhouse rearing cages averaged 6 to 9 days. Duration of pupation in the environmental chamber averaged 4-7 days. Harvesting cocoons from the greenhouse rearing cages and placing them in gelatin capsules took an average of 45 minutes.
Table 13 shows the sex ratio of *C. rubecula* from June 1987 to March 1989. Sex ratios were recorded for 3550 cocoons; 1165 were female and 2114 were male (a 0.56:1.0 sex ratio).

To increase production, the number of plants per oviposition cage could be increased to a maximum of eight, with twelve *C. rubecula* females provided. Six broccoli plants with parasitized larvae and an equal number of uncolonized plants could be placed in the greenhouse rearing cage. Multiples of the oviposition and rearing cages could be used for mass production.

**Adaptations for rearing Cotesia glomerata (L.).** The same ovipositional setup can be used for *C. glomerata* (Hymenoptera: Braconidae), a gregarious larval parasite of *P. rapae*. However, a single *P. rapae* larva parasitized by *C. glomerata* ingests more than ten times the leaf area compared to a larva parasitized by *C. rubecula* (Parker 1973). Thus, only two plants with the same number (35) of *P. rapae* larvae per plant are offered to six female *C. glomerata* in the ovipositional cages. Greenhouse rearing cage techniques are identical, with the exception of supplying six to eight additional plants per cage to allow parasitized larvae to complete development. Cocoon harvest, separation, and encapsulation took 65 minutes for four *C. glomerata* cocoon masses averaging 30 cocoons each.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Generations</td>
<td>42</td>
</tr>
<tr>
<td>Total Cocoons</td>
<td>3550</td>
</tr>
<tr>
<td>Female</td>
<td>1165</td>
</tr>
<tr>
<td>Male</td>
<td>2114</td>
</tr>
<tr>
<td>No adult emergence</td>
<td>271</td>
</tr>
<tr>
<td>Female/Male Ratio</td>
<td>0.56</td>
</tr>
<tr>
<td>Time/100 Cocoons</td>
<td>4.7 h</td>
</tr>
<tr>
<td>Ave. Cocoons/Cage</td>
<td>84.5</td>
</tr>
</tbody>
</table>
Vita

Richard C. McDonald was born on January 5, 1956 in Sedalia, Missouri. After graduating from Warsaw High School in 1974, he began undergraduate studies at the University of Missouri-Columbia. Upon the death of his father in 1978, he left the university to become an outside salesman for Rockbottom's Home Care Center in Columbia, Missouri. In 1981, he reentered the University of Missouri-Columbia and completed a B.S. in Agriculture majoring in IPM in 1982. He then became a laboratory technician at the USDA Biological Control of Insects Research Laboratory in Columbia, Missouri until 1983, when he began work on his M.S. in the Department of Entomology at the University of Missouri-Columbia. He married Kathryn Cahow on May 27, 1984. After completing his M.S. and receiving the Phillip C. Stone Scholarship Award for Outstanding Research in Entomology and the C.V. Riley Award of Merit, he entered VPI & SU in April 1986 to pursue a Doctor of Philosophy degree in Entomology.

The author is a member and past president of the W.B Alwood Entomological Society and the Gamma Sigma Delta honorary society. He was awarded the David R. Spence Scholarship in 1987.