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The Biology and Ecology of the Yellowmargined Leaf Beetle, Microtheca Ochroloma Stal, (Coleoptera: Chrysomelidae) on Crucifers.

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THE BIOLOGY AND ECOLOGY OF THE YELLOWMARGINED LEAF BEETLE, Microtheca ochroloma Stål, (COLEOPTERA: CHRYSOMELIDAE) ON CRUCIFERS

A Dissertation
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirement for the degree of Doctor of Philosophy in The Department of Entomology

by
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>SECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION AND LITERATURE REVIEW**
   - Taxonomy | 6 |

2. **COLONY ESTABLISHMENT AND MAINTENANCE** | 8 |

3. **MULTI-GENERATION SURVIVORSHIP, LIFE CYCLE AND DEVELOPMENTAL BIOLOGY OF THE YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) ON CRUCIFERS**
   - Introduction | 14 |
   - Materials and Methods | 17 |
   - Results | 24 |
   - Discussion | 27 |

4. **FECUNDITY AND LONGEVITY OF THE YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) ON CRUCIFERS**
   - Introduction | 36 |
   - Materials and Methods | 39 |
   - Results | 42 |
   - Discussion | 44 |
5. FEEDING PREFERENCE OF THE YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) AMONG CRUCIFERS ...............55
   Introduction .......................................55
   Materials and Methods ..............................58
   Results ............................................62
   Discussion .........................................66

6. SPATIAL DISPERSION OF THE YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) ON MUSTARD ..................71
   Introduction ......................................71
   Materials and Methods .............................74
   Results ...........................................78
   Discussion ........................................80

7. INSECTICIDE SUSCEPTIBILITY AND DETOXIFYING ENZYMES OF THE YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSMELIDAE) ........................................91
   Introduction .......................................91
   Materials and Methods ..............................94
   Results ............................................99
   Discussion ........................................101

REFERENCES CITED ........................................110

VITA ......................................................126
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1. Multi-generation survivorship of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip</td>
<td>28</td>
</tr>
<tr>
<td>3.2. Pupal weight of the yellowmargined leaf beetle reared over four generations on cabbage, collard, mustard, radish and turnip</td>
<td>29</td>
</tr>
<tr>
<td>3.3. Life cycle and duration of development of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip</td>
<td>30</td>
</tr>
<tr>
<td>3.4. The effect of temperature on duration of development of immature yellowmargined leaf beetle</td>
<td>31</td>
</tr>
<tr>
<td>4.1. Morphometric data of a laboratory population of the yellowmargined leaf beetle maintained on cabbage, collard, mustard, radish and turnip</td>
<td>48</td>
</tr>
<tr>
<td>4.2: Pearson's correlation coefficients for the relationship of fecundity to length, weight and longevity of a laboratory population of the yellowmargined leaf beetle maintained on cabbage, collard, mustard, radish and turnip</td>
<td>49</td>
</tr>
<tr>
<td>5.1. Sequence of replication of the feeding preference studies of the yellowmargined leaf beetle for the foliage of cabbage, collard, mustard, radish and turnip</td>
<td>61</td>
</tr>
</tbody>
</table>
5.2. Feeding preference of first and third instar larval yellowmargined leaf beetle as determined by number of larvae feeding on the foliage of cabbage, collard, mustard, radish and turnip. 64

5.3. Feeding preference of third instar larvae and adult yellowmargined leaf beetle for crucifer plants as determined by leaf consumption (mg). 65

6.1. Within-plant count of the distribution of yellowmargined leaf beetle life stages on mustard at the St. Gabriel Research Station, 8/92 to 1/93. 81

6.2. Between-plant count of the distribution of yellowmargined leaf beetle life stages on three plots of mustard at the Louisiana Agricultural Experiment Stations. 82

6.3. Taylor’s power law and Iwao’s patchiness regression statistics for yellowmargined leaf beetle life stages taken from mustard on plot one at the St. Gabriel Research Station, Iberville Parish, LA, in the fall of 1992. 84

6.4. Taylor’s power law and Iwao’s patchiness regression statistics for yellowmargined leaf beetle life stages taken from mustard on plot two at the St. Gabriel Research Station, Iberville Parish, LA, in the spring of 1993. 85

6.5. Taylor’s power law and Iwao’s patchiness regression statistics for yellowmargined leaf beetle life stages taken from mustard on plot at the Burden Research Station, East Baton Rouge Parish, LA in the fall of 1995. 86
6.6. Chi-square goodness of fit for the negative binomial distribution of yellowmargined leaf beetle life stages collected from mustard at the Burden Research Station, fall 1995.........................87

6.7. Estimates of the values of $k$ for the negative binomial distribution of yellowmargined leaf beetle life stages taken from mustard at the Burden Research Station, East Baton Rouge Parish, LA in the fall of 1995........................................88

7.1. Susceptibility (LD$_{50}$) of yellowmargined leaf beetle larvae maintained on collard and turnip to carbaryl, esfenvalerate and malathion.........................102

7.2. The activities of glutathione S-transferases and esterases enzymes of yellowmargined leaf beetle larvae fed collard and turnip.........................104
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Ventral view of the last abdominal segment of pupal yellowmarginedin leaf beetle showing sexually dimorphic character</td>
<td>13</td>
</tr>
<tr>
<td>4.1</td>
<td>Fecundity of the yellowmarginedin leaf beetle on cabbage, collard, mustard, radish and turnip</td>
<td>45</td>
</tr>
<tr>
<td>4.2</td>
<td>Longevity of the yellowmarginedin leaf beetle on cabbage, collard, mustard, radish and turnip</td>
<td>46</td>
</tr>
<tr>
<td>4.3</td>
<td>Daily fecundity of the yellowmarginedin leaf beetle on cabbage, collard, mustard, radish and turnip</td>
<td>47</td>
</tr>
<tr>
<td>5.1</td>
<td>Five leaf arrangements of crucifer plants used in the feeding preference studies of yellowmarginedin leaf beetle for the foliage of cabbage, collard, mustard, radish and turnip</td>
<td>60</td>
</tr>
</tbody>
</table>
ABSTRACT

The biology and ecology of the yellowmargined leaf beetle, *Microtheca ochroloma* Stål, were studied on cabbage, *Brassica oleracea* var *capitata* L., collard, *B. oleracea* var *acephala* L., mustard, *B. juncea* Cosson, turnip, *B. rapa* L., and radish, *Raphanus sativus* L. The life cycle of the beetle consists of an egg stage, four larval instars, prepupal, pupal and adult stages. There were no significant differences in the effect of host plant on duration of development of immature beetles ($p = 0.3353$). The mean duration of development from oviposition to adult emergence ranged from 26.6 d on turnip to 27.5 d on cabbage. There was however a significant effect of host plant on multi-generation survivorship. Beetles reared on cabbage did not survive beyond the second generation.

With respect to adult beetles, significant differences were found in the effect of host plant on fecundity ($p = 0.0057$) and longevity ($p = 0.0001$). The mean fecundity of females was significantly higher for beetles maintained on turnip ($490.74 \pm 116.04$) than for those maintained on collard ($198.85 \pm 28.94$). There were no significant differences in the mean fecundities of females
maintained on cabbage (271.25 ± 39.11), mustard (424.95 ± 46.39) and radish (440.05 ± 50.09). Beetles fed radish lived significantly longer than beetles fed each of the other host plants. There were no significant differences in the longevity of male and female beetles by host plant.

In choice tests for feeding preference, both the third larval instar and adult beetles showed strong preference for the foliage of turnip and mustard. Collard and cabbage were least preferred. Susceptibility of beetle larvae to insecticides was esfenvalerate > carbaryl > malathion. There were no significant differences in the effects of host plant on susceptibility of the larvae to the insecticides. There was however a 10-fold difference in the activities of glutathione S-transferases enzymes for beetle larvae fed collard and those fed turnip.

Spatial distribution studies revealed that both immature and adult beetles have aggregated spatial patterns on field planted mustard.
The yellowmargined leaf beetle, Microtheca ochroloma Stål, is a serious foliar pest of cruciferous crops in Louisiana (Oliver and Chapin 1983). This beetle, indigenous to South America, was accidentally introduced into the United States around 1945. The first specimen was intercepted on grapes from Argentina by port inspectors at New Orleans in 1945 (Chamberlin and Tippins 1948). A field infestation was recorded for the first time on young cabbage plants, Brassica oleracea var capitata L., at Springhill, Alabama on March 20, 1947 by Chamberlin and Tippins (1948). In later months, surveys around Mobile, Alabama revealed county-wide infestations on collard, B. oleracea var acephala L., mustard, B. juncea Cosson, turnip, B. rapa L., and radish, Raphanus sativus L. In addition, the beetle has also been reported to feed on watercress, Nasturtium officinale R. Brown, an aquatic cruciferous plant in Florida (Woodruff 1974).

In South America, the beetle has been recorded from Argentina, Brazil, Chile and Uruguay (Jolivet 1950, Anonymous 1962). Its geographical distribution in the US
is not completely known but is at present restricted to the Southeast. Infestations have been reported from Louisiana (Oliver and Chapin 1983), Florida (Woodruff 1974), Alabama (Chamberlin and Tippins 1948), Mississippi (Haeussler 1951) and Texas (Balsbaugh 1978). There have also been unconfirmed reports of infestations in Georgia, and North and South Carolina.

Both adults and larvae damage the hosts by feeding on the foliage. The adults make small, irregularly shaped holes in the leaves and feed upon leaf margins (Chamberlin and Tippins 1948). The larvae, like those of many Chrysomelidae, feed gregariously when newly hatched (Jolivet 1950). *Microtheca ochroloma* is a cool season pest developing several generations in the field, from early in the winter, through the beginning of summer. Its whereabouts during the hot summer months is not yet known. It is known, however, that the high summer temperature in the southeastern United States does not favor the growth in the field of the crucifer hosts on which the beetle feed. It may aestivate during this period (Anonymous 1962, Oliver and Chapin 1983), or perhaps move onto alternate hosts. The alternate host theory is supported by reports of some
authors that have found the beetle to be associated with plants other than crucifers. Gentry (1954) reported that the beetle was found feeding on Irish potato in Marengo county, Alabama. Rohwer et al. (1953) found a considerable number of the beetles feeding on wild plants of the primrose family. The beetle was also collected by Oliver and Chapin (1983) on Lepidium virginicum L., Rumex sp. and on clovers and vetch. There is thus the need for an intensive search for the yellowmargined leaf beetle during the summer months in order to determine its whereabouts. The fact that the beetle can be reared in the laboratory all year round without a diapause also supports the alternate host theory. Microtheca ochroloma can continue its normal activities all year round as long as a source of food is available.

In Louisiana, mustard, turnips and collards, collectively called ‘greens’, are fall and winter vegetable crops. According to Boudreaux et al. (1990), greens are one of the more profitable vegetable crops in the state. The small initial investment needed, coupled with the short growing season, result in a quick turnover in crops. In 1994, gross farm income from commercial vegetable
production in the state was estimated to be $40 million (Parish et al. 1995). The impact of the yellowmargined leaf beetle on greens production in the state has not yet been evaluated. Field observations and grower reports in East Baton Rouge Parish and other parishes in Louisiana have indicated that this insect might be the key pest on mustard and turnip. Field plantings of these crops are quickly invaded and a very high population can develop within a short time if adequate control measures are not taken. There is no known natural enemy of the beetle in the United States at present. Since greens are grown and harvested primarily for their foliage, insect feeding damage like that caused by the yellowmargined leaf beetle makes them unsightly for sale. There is no doubt that the beetle negatively impacts production of greens in the state.

Little is known about the biology and ecology of *M. ochroloma* despite its pest status and its statewide distribution in Louisiana. Most of the reports on this beetle in the literature have focused on its geographical distribution in the US and its host association in the field (Chamberlin and Tippins 1948, Haeussler 1951,
The first published report on the biology of the beetle was by Oliver and Chapin (1983). They studied the fecundity, longevity and developmental biology of the beetle on turnip under laboratory conditions. Though their report revealed some information about the biology of the beetle on turnip, there is the need for more studies on the performance of the beetle on many of the crucifer host plants on which the beetle has been found in the field. Such studies will reveal valuable information about the suitability of each plant as a host for the beetle. This information will be useful in the identification of host(s) that exhibit resistance to the beetle and possibly the mechanism of resistance. Plant resistance to insects is a control tactic used in insect pest management (Adkisson and Dyck 1980).

The broad objective of my research was to study the biology and ecology of the beetle on cabbage, collard, mustard, radish and turnip. Specific objectives were to study longevity and fecundity, determine feeding preference, describe developmental sequence and enumerate the length of time for development. In addition, bioassays
were planned to test the susceptibility of the beetle to selected insecticides. A study of spatial dispersion patterns of immature and adult beetles in the field was also undertaken.

Taxonomy

The genus *Microtheca* Stål was treated extensively by Jolivet (1950). *Microtheca* was first mentioned in the catalog by Dejean (1837) and described by Stål (1860). Weise (1915), using the following morphological characters, placed the genus *Microtheca* in the tribe Timarchini: closed anterior coxal cavities and claws simple or slightly curved at the base. However, Chen (1934), cited by Balsbaugh (1978), placed *Microtheca* in the tribe Entomoscelini in the subfamily Chrysomelinae and the family Chrysomelidae. There are fourteen species in this genus and all were confined in distribution originally to South America (Jolivet 1950). The species of *Microtheca* are *M. columbiana* Steinheil in Colombia; *M. boliviana* Achard, *M. orophila* Jolivet and *M. nitens* Bechyne in Bolivia; *M. bechynei* Jolivet, *M. semilaris* Stål and *M. freyi* Jolivet in Peru; *M. ochroloma* Stål, *M. semilaris* Stål and *M. picccitaris* Stål in Brazil and *M. punctigera* in Argentina.
Other species are *M. planicollis* Bechyne, *M. picea* Guérin, *M. parvula* Bechyne and *M. vittata* Weise.

The two species of *Microtheca* that have been reported in the US at present are *M. ochroloma* and *M. picea*. *Microtheca ochroloma* seems to be the more widely distributed. *Microtheca picea* has been reported only from Texas and Alabama (Balsbaugh 1978) and Louisiana (Oliver and Chapin 1983). Balsbaugh (1978) provided a key to separate *M. ochroloma* from *M. picea*. He described *M. ochroloma* as being 4.2 to 6.0 mm long and having a dark brown dorsum with elytra margined with dull yellow color. Each elytron has four striae of very large punctures that are distinct beyond the middle of the elytron. On the other hand, *M. picea* is said to be 4.1 to 5.2 mm long with a uniform dark brown dorsum and each elytron has 7 to 9 striae of small punctures. The punctures are distinct to about basal half and are evanescent caudad.

Woodruff (1974) gave a short description of adult beetles. It is about 5mm long, bronzy black to dark brown in color with yellow margins around the elytra (hence the common name 'yellowmargined' leaf beetle). The third tarsal segment is bilobed and there are four prominent rows of punctures on each elytron.
CHAPTER 2

COLONY ESTABLISHMENT AND MAINTENANCE

A colony of the yellowmargined leaf beetle was started in the laboratory in the fall of 1992 from field collected specimens. A small field plot was planted with mustard (cv 'Florida broadleaf') to attract the beetle at the St. Gabriel Research Station, Louisiana State University Agricultural Experiment Station, Iberville Parish, LA. After about five weeks, the plants were well established and had a sizable infestation of M. ochroloma. Beetles were collected at five weekly intervals between September 23 and October 30, 1992. The number of beetles brought into the laboratory by life stages were 18 eggs, 167 'small' larvae (first and second instars), 86 'large' larvae (third and fourth instars), 7 pupae and 13 adult specimens. In the spring of 1993, more beetle specimens were collected from a second mustard plot at the same location on November 13, 1992, January 18 and 25, 1993. This consisted of 19 eggs, 43 'small' larvae, 11 'large' larvae and 14 adult specimens.

In the laboratory, specimens were sorted by life stage (egg, larva, pupa and adult) and placed on top of a
moistened white filter paper (9 cm diameter, VWR., FILTER PAPERS, QUALITATIVE, GRADE 413) in circular petri dishes (100 X 15 mm). The larvae were sorted by size, placed in groups of ten in dishes and supplied with mustard foliage obtained from field plantings. The adults were also placed in groups in dishes and maintained on mustard foliage. The dishes were arranged on trays in a growth room maintained at 20°C, 70% RH and 14L:10D photophase. At the initial stages, because of the difficulties in separating the sexes, adult beetles were placed in groups so that when males and females pair up, the paired beetles were separated into petri dishes. Although Oliver and Chapin (1983) separated adult males from females by their decurved posterior abdominal sternum, this character was very difficult to apply in separating the sexes. However with time, I was able to separate the sexes at the pupal stage with a high degree of accuracy. Though female pupae are bigger and heavier than males, size alone cannot be used to separate the sexes accurately. The morphological character that was used to separate male from female pupae was a pair of knob-like structures located on the ventral side of the terminal abdominal segment of female pupae (Figure 2.1).
This structure is absent in the males. The accuracy of this method was verified each time by placing some male and female pupae individually in dishes and observing that when the adult females emerged, they laid eggs. After learning how to sex the pupae, both sexes were separated at every pupal stage. When the adults emerged, males and females were paired in petri dishes to lay viable eggs. Using a camel hair brush, these eggs were collected daily and placed in groups of ten in petri dishes. When the larvae eclosed, they were maintained on mustard foliage. Larvae were reared until adult emergence and this cycle was repeated several times until the colony became properly established.

Since the broad objective of my research was to study the biology of the beetle on five crucifer host plants, it became necessary to establish and maintain separate colonies of the beetle on each host. To ensure that insecticide and disease free foliage was available to maintain beetle colonies, plants were raised in the greenhouse. The varieties were 'Early round Dutch' cabbage, 'Georgia' collard, 'Florida broadleaf' mustard, 'Scarlet globe' radish, and 'Purple top white globe'
turnip. These are the most popular varieties of each plant among Louisiana growers. In the process of planting, seeds were pre-germinated on moistened filter paper in petri dishes in the laboratory. In about five days, the seedlings were ready to be transplanted. Seedlings were transplanted into Jiffypots® (about 6.4 cm square) filled with Jiffymix®. Small holes were drilled at the bottom of each pot to ensure proper drainage. The pots were arranged in trays and the trays were placed on benches in the greenhouse. The plants were watered as needed and fertilized on alternate days using Miracle-gro® at the rate of one spoonful (about 18.123g) in about 7.6 liters of water. Foliate from each plant was ready to be harvested about three weeks after the seedlings were transplanted. New plantings were made every ten days to ensure that young foliage was available to feed the beetles.

The foliar nitrogen content of each plant was determined by the Kjeldahl method (see Bradstreet 1965) on three separate foliage samples of young leaves that were randomly selected at three different plantings. This determination was made by personnel of the Louisiana
Department of Agricultural Chemistry. The percent foliar nitrogen of each plant was 6.89 ± 0.35 on cabbage, 7.29 ± 0.58 on collard, 6.53 ± 0.28 on mustard, 6.50 ± 0.15 on radish and 6.48 ± 0.14 on turnip. Analysis of variance revealed that there were no significant differences in the foliar nitrogen content of each plant (F = 2.90, df = 4, P = 0.0932).
Figure 2.1. Ventral view of the last abdominal segment of pupal yellowmargined leaf beetle showing sexually dimorphic character.
CHAPTER 3

MULTI-GENERATION SURVIVORSHIP, LIFE CYCLE AND DEVELOPMENTAL BIOLOGY OF THE YELLOWMARGINED LEAF BRETLE (COLEOPTERA: CHRYSMELIDAE) ON CRUCIFERS

Introduction

In endopterygote insects, development is a sequence of distinct stages: egg, larval-feeding instars, a pupal instar and the adult (Engelmann 1984). The duration of each stage, changes in size and form, and the probability of survival are determined by complex interactions of internal growth-regulating mechanisms with environmental conditions and the quality of food. The amount, rate and quality of food consumed by the larvae influence their survival, growth rate, developmental time and final body weight (Scriber and Slansky 1981). For phytophagous insects, the quality of a host plant as food is affected by variation in the amount of basic nutrients and non-nutritional compounds (e.g. allelochemicals) (Slansky 1982). Foliar nitrogen has been suggested to be one of the most important nutritional factors (Mattson 1980, Myers and Post 1981). The non-nutritional compounds include the allelochemicals, compounds that were evolved by many plants
as a means of defense to phytophagous insects (Dethier 1954, Fraenkel 1959, Ehrlich and Raven 1964, Whittaker and Feeny 1971). However, some workers have suggested that moisture content and leaf toughness might be as important as foliar nitrogen and defensive chemicals in determining the quality of a host plant as food (Feeny 1970, Slansky and Feeny 1977, Scriber 1979).

Temperature and photoperiod are the two most important environmental factors affecting the development of insects (Scriber and Slansky 1981). Due to water loss problems, the maintenance costs of poikilothermic animals generally increase with increasing temperature (Precht et al. 1973). There is, however, a range of temperatures at which the development and survival of an insect is at optimum. In the elm leaf beetle, Pyrrhalta luteola (Muller), for instance, developmental duration decreased significantly with increasing temperature (King et al. 1985). The western corn rootworm, Diabrotica virgifera virgifera Leconte, has optimum development and survival between 21 - 30°C (Jackson and Elliot 1988). In addition to temperature optima, fluctuating or cycling temperature regimes favor growth and development relative to constant

Though field populations of *Microtheca ochroloma* have been reported on cabbage, collard, mustard, radish and turnip, the developmental biology has been studied and reported only on turnip (Oliver and Chapin 1983). In the laboratory, using field-planted turnip to maintain the beetle at 27°C, they reported that the insect develops from egg through three larval instars, a prepupa, pupal and adult stages, lasting about 23 days. While this study revealed some information about the developmental biology of the beetle on turnip, there is the need for similar studies on some of the other host plants as well. A comparative study of the developmental biology of the beetle on these plants might reveal information about the possibility of some of the plants expressing resistance to
the beetle. Resistance may be manifested as larval mortalities, failure of larvae to pupate and longer time for development. In this chapter, the objectives were to describe the developmental sequence, enumerate the duration of development, determine the effect of plant and temperature on duration of development and determine the effect of plant on multi-generational survivorship.

Materials and Methods

**Colony establishment and multi-generation survivorship.** In order to study aspects of the biology of yellowmargined leaf beetle listed above, it was necessary to establish and maintain separate colonies of the beetle on cabbage, collard, radish and turnip in addition to the existing mustard colony. Eggs collected from female beetles in the mustard colony were used to start separate colonies of the beetle on each of the other host plants. Beetles were reared continuously, on each host plant, one generation after another so as to have an idea of the effect each plant has on multi-generation survivorship and pupal weight. To begin the study, 200 eggs were collected from beetles in the mustard colony and placed in groups of ten in petri dishes. The bottom part of the dishes were lined
with a single piece of filter paper and kept moist by adding distilled water as needed. The eggs were randomly divided equally into five groups (40 eggs in each group) and each group was randomly assigned to the five host plants. Each time eggs were collected and the larvae reared on foliage of the other four host plants, fresh eggs were collected and reared on mustard as well. Dishes were arranged on trays by host plant and labeled accordingly. The trays were placed on benches in a growth room maintained at 20°C, 70% RH and 14L:10D photophase. When the eggs hatched, the larvae were maintained on the foliage of the assigned plant until pupation. At pupation, the pupae were sexed using the character described in Chapter 2 (See Figure 2.1) and weighed. The number of adults emerging from these pupae were counted and recorded. Adult males and females were paired in groups in petri dishes and supplied with appropriate foliage. These were the F1 adults on each host plant (except mustard). In order to have a sizable number of beetles in each colony, the cycle of rearing from egg to adult was repeated again with a second batch of 200 eggs collected from beetles in the mustard colony.
From each colony, eggs laid by the F1 adults were collected daily and allowed to hatch until a maximum number of 100 first instars were obtained. These larvae were placed in groups of ten in petri dishes and maintained on appropriate foliage until pupation. The pupae were sexed and weighed. The number of adults emerging from these pupae were also counted and recorded. These were the F2 adults in each colony (except mustard). These adults were also paired by sexes to lay viable eggs, which were collected daily. At eclosion, 100 first instars were randomly selected by host plant and maintained on the appropriate host foliage until pupation. The pupae were sexed and weighed as before. At this juncture, an interesting development was observed. A substantial number of larvae maintained on cabbage failed to pupate. As a result, a second batch of 100 first instars from eggs obtained from the F2 cabbage adults were reared on cabbage foliage till pupation and adult emergence. On the other host plants, beetle rearing was continued beyond the third to the fourth generation.

**Life cycle and duration of development studies.** The life cycle and duration of development of immature beetles from
oviposition to adult emergence were studied on the five crucifer host plants at 20 ± 1°C, 50% RH and 14L:10D photophase. A parallel experiment investigating the effect of temperature on duration of development was set up at 25 ± 2°C, 80% RH and 14L:10D photophase using insects raised on turnip only. Eggs collected from beetles in the mustard colony were used to start separate beetle colonies on each of the other four host plants. Each time eggs were collected and the larvae reared on the foliage of the other plants, fresh eggs were collected and the larvae reared on mustard foliage as well. The rearing procedure was as described above. Beetles were reared on each host plant for one full generation before the study began. The F1 adults in each host plant colony were paired by sex, placed in groups of ten in petri dishes and fed on appropriate foliage until the females started laying eggs. It was these eggs that were collected and used in the study. To begin the study, at about 1000 hours, designated the zero hour, all the eggs in each colony were collected and discarded. The filter papers were replaced and fresh foliage supplied. Twenty four hours after this exercise, fresh eggs were collected, placed in groups of ten on top
of moistened filter papers in petri dishes and labeled according to host plant. Ten eggs were randomly selected from the pool of eggs by host plant and placed individually on top of moistened filter papers in petri dishes. The dishes were randomly assigned numbers from 1 to 10. The numbered dishes and the ones containing the pool of eggs were placed in plastic storage boxes (10 X 15 X 30 cm) and covered. To maintain high humidity inside each box, the bottoms were filled with water, one cm in depth, above which a piece of cardboard was placed and supported with inverted petri dish covers (5.5 cm in diameter). The boxes were arranged in the incubator (about 91 cu. cm Hotpack) and maintained at a temperature of 20 ± 1°C, 50% RH and 14L:10D photophase. A separate experiment was set up using eggs collected from beetles in the turnip colony in an incubator maintained at 25°C, 80% RH and 14:10 L:D photophase. Dishes were checked daily between 1000 and 1200 h to monitor biological activities (egg hatching, molting, pupation, mortality etc.). The period of time between oviposition and egg hatching was noted and recorded by host for each experimental unit. The percent egg hatching was calculated from all eggs collected by host
plant. On hatching, larvae in the numbered dishes were supplied with a leaf disc (15 cm²) of appropriate plant. Dishes were checked carefully for larval molting. The presence of an exuvium (head capsule and/or cast larval skin) was used as a sign of molting. At each molt, the leaf discs and filter papers were changed. At pupation, the pupae were sexed and weighed. Daily observations continued until adult emergence. The number of days between pupation and adult emergence was also noted and recorded.

**Days to the beginning of oviposition.** On emergence, adult beetles were supplied with appropriate foliage, and checked daily until females began oviposition. The number of days between adult emergence and the beginning of oviposition were noted for each female, by host. This is the days to the beginning of oviposition. As soon as a female started laying eggs, it was removed from the experiment. The experiment was terminated after all the females began ovipositing.

The experiment was replicated three times. The experimental unit was increased from 10 to 20 at the second and third replication. At each replication, rearing was
started afresh from eggs obtained from beetles in the mustard colony.

**Data analysis.** The number of larvae that survived to pupate and the number of adults emerging from these pupae were calculated as percent pupation and adult emergence respectively by host plant at each generation. Pupal weight data were analyzed for the effect of host plant and generation as analysis of variance (5 by 4 factorial) with a completely randomized design ($\alpha = 0.05$ [SAS Institute 1989]). Means separation for the effect of host plant and generation on pupal weight was done using Tukey's test. Means of pupal weight by sex were compared using a Students t-test.

For the developmental biology studies, percentage egg hatching was calculated from the total number of eggs collected by host plant. The length of time spent in each life stage (egg, larval instars, prepupa and pupa) was calculated for each experimental unit by host plant. From these, the total duration of development (in days) from oviposition to adult emergence was calculated. The duration of development and days to oviposition data were analyzed separately as a randomized block design using the
general linear model (GLM) procedure (model developmental
time, days to oviposition = plant, sex, block, plant by
block). Means separation for developmental time and days
to oviposition variables were done using Tukey's test.
Means separation for the effect of temperature on the
variables were done using a Student's t-test.

Results

Multi-generation survivorship and pupal weight. The
yellowmargined leaf beetle was reared continuously and
survived for several generations on mustard foliage in the
laboratory. In addition, the beetle was reared
continuously and survived for four generations on collard,
radish and turnip. However, on cabbage, a significant
number of the third generation larvae did not survive to
pupate (Table 3.1). The few that pupated metamorphosed
into adults that had reduced fitness. None of the females
among these adults laid eggs. The percent pupation and
adult emergence by host plant are shown in Table 3.1.
There were significant effects of host plant on pupal
weight ($F = 14.39, df = 4, P = 0.0001$), sex on pupal weight
($F = 1994.26, df = 1, P = 0.0001$) and the pupal weights
were significantly different between generations
(F = 28.81, df = 3, P = 0.0001). Larvae maintained on turnip resulted in significantly heavier pupae than those maintained on collard, radish and cabbage (Table 3.2). There were no significant differences in the weight of pupae resulting from larvae maintained on turnip and mustard. With regards to generation, first generation pupae were the heaviest. Female pupae were significantly heavier than males. On average, the weight of female and male pupae were 10.90 and 7.97 mg, respectively.

Developmental biology studies. As shown in Table 3.3, the life cycle of the beetle consists of an egg stage, four larval instars, prepupal, pupal and adult stages. A fifth larval instar was recorded on some of the host plants. The number and percentage of larvae that went through the fifth instar were 2 of 46 (4.3%) on cabbage, 3 of 46 (6.5%) on collard, 2 of 47 (4.3%) on mustard and 2 of 44 (4.5%) on turnip. The eggs are bright orange in color, elongate and are laid either singly or in batches. The larva is brown in color with a dark head capsule and has many circular rows of setigerous tubercles on the body trunk. There are six stemmata on either side of the head which are clearly visible under a dissecting microscope immediately after a
molt before the cuticle hardens. There is a prepupal stage lasting between two and three days and characterized by a cessation of feeding, wandering behavior and eventually the larva ceases movement when a suitable place to pupate is found. It spins a cocoon around itself and remains inactive inside the cocoon. The pupa is light brown or tan in color, exarate and displays most of the adult features. Laterally the body bears setae and there is a distinctive sexually dimorphic structure that was helpful in separating the sexes (Figure 2.1).

The mean percent egg hatching were 95.7 ± 0.3 on cabbage, 96.3 ± 0.3 on collard, 97.3 ± 0.3 on mustard, 96.7 ± 0.7 on radish, and 97.0 ± 0.6 on turnip. The duration of development of each life stage, the total duration of development from oviposition to adult emergence and days to the beginning of oviposition by host plant are reported in Table 3.3. There were no significant effects of host plant on the mean duration of development from oviposition to adult emergence ($F = 1.34$, $df = 4$, $P = 0.3353$). On the average, the duration of development ranged from between $26.47 ± 0.14$ d for beetles maintained on turnip to $27.63 ± 0.14$ d for those maintained on cabbage (Table 3.3). The
duration of development was not significantly different for male and female beetles ($F = 0.03$, df = 1, $P = 0.8764$). However, there were significant differences in the effects of host plant on days to the beginning of oviposition by females ($F = 6.64$, df = 4, $P = 0.0470$). On the average, adult females started laying eggs about four days after emergence (Table 3.3). With respect to temperature, beetles maintained at 25°C developed significantly faster than beetles maintained at 20°C ($F = 16.54$, df = 1, $P = 0.05$). Beetles maintained at 25°C required about 19 days for development while those maintained at 20°C required about 27 days (Table 3.4). Unlike host plant, however, temperature did not have any effect on days to the beginning of oviposition ($F = 0.10$, df = 1, $P = 0.804$).

With respect to length of development of each life stage, neither host plant nor temperature had any significant effect.

Discussion

This research demonstrated that Microtheca ochroloma can survive and complete its development on cabbage, collard, mustard and radish. Oliver and Chapin (1983) had earlier reported similar information for the beetle on
Table 3.1. Multi-generation survivorship of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>Generations</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>Pupa</td>
<td>Adult</td>
<td>Pupa</td>
<td>Adult</td>
</tr>
<tr>
<td>Cabbage</td>
<td>85</td>
<td>79</td>
<td>79</td>
<td>71</td>
</tr>
<tr>
<td>Collard</td>
<td>90</td>
<td>81</td>
<td>85</td>
<td>78</td>
</tr>
<tr>
<td>Mustard</td>
<td>96</td>
<td>92</td>
<td>91</td>
<td>88</td>
</tr>
<tr>
<td>Radish</td>
<td>91</td>
<td>88</td>
<td>89</td>
<td>86</td>
</tr>
<tr>
<td>Turnip</td>
<td>95</td>
<td>89</td>
<td>90</td>
<td>85</td>
</tr>
</tbody>
</table>

*a % pupation

*b % adult emergence
Table 3.2. Pupal weight of the yellowmargined leaf beetle reared over four generations on cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>Plant</th>
<th>1st generation</th>
<th>2nd generation</th>
<th>3rd generation</th>
<th>4th generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male^b</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Cabbage</td>
<td>7.81 ± 11.02</td>
<td>7.14 ± 10.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>0.15</td>
<td>0.19</td>
<td>0.23</td>
</tr>
<tr>
<td>Collard</td>
<td>8.41 ± 11.13</td>
<td>7.76 ± 10.95</td>
<td>8.19 ± 10.18</td>
<td>8.09 ± 10.49</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.19</td>
<td>0.19</td>
<td>0.25</td>
</tr>
<tr>
<td>Mustard</td>
<td>8.86 ± 11.6</td>
<td>8.07 ± 11.19</td>
<td>7.77 ± 10.64</td>
<td>8.02 ± 10.72</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.18</td>
<td>0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>Radish</td>
<td>8.54 ± 11.44</td>
<td>8.19 ± 10.84</td>
<td>8.14 ± 10.48</td>
<td>8.04 ± 10.66</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.21</td>
<td>0.15</td>
<td>0.19</td>
</tr>
<tr>
<td>Turnip</td>
<td>8.68 ± 11.92</td>
<td>8.07 ± 11.33</td>
<td>7.96 ± 11.43</td>
<td>7.70 ± 11.09</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.20</td>
<td>0.19</td>
<td>0.14</td>
</tr>
</tbody>
</table>

^a indicates pupal weight significantly affected by host plant (α = 0.05); Tukey’s test.

^b indicates significant differences between male and female pupal weight (α = 0.05); Tukey’s test.
Table 3.3. Life cycle and duration of development of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>Life Stages</th>
<th>Cabbage</th>
<th>Collard</th>
<th>Mustard</th>
<th>Radish</th>
<th>Turnip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of development (d) (Mean ± SE)</td>
<td>7.8 ± 7.7 ± 7.8 ± 7.9 ± 7.8 ±</td>
<td>0.08 ± 0.09 ± 0.06 ± 0.06 ± 0.06 ±</td>
<td>(48) ± (49) ± (47) ± (49) ± (48) ±</td>
<td>3.0 ± 2.6 ± 2.7 ± 2.7 ± 3.1 ±</td>
<td>0.07 ± 0.07 ± 0.09 ± 0.09 ± 0.11 ±</td>
</tr>
</tbody>
</table>

\(n = 50\); Numbers in parentheses are the experimental unit.

\(a\) No significant effect of plant on mean duration of development (\(\alpha = 0.05\)); Tukey's test.

\(b\) Days to the beginning of oviposition by the females.
Table 3.4. The effect of temperature on duration of development of immature yellowmargin leaf beetle.

<table>
<thead>
<tr>
<th>Life Stages</th>
<th>20°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>7.8 ± 0.06 (48)</td>
<td>5.7 ± 0.07 (50)</td>
</tr>
<tr>
<td>1st instar</td>
<td>3.1 ± 0.11 (48)</td>
<td>2.2 ± 0.07 (49)</td>
</tr>
<tr>
<td>2nd instar</td>
<td>1.9 ± 0.09 (47)</td>
<td>1.4 ± 0.07 (48)</td>
</tr>
<tr>
<td>3rd instar</td>
<td>2.2 ± 0.08 (47)</td>
<td>1.4 ± 0.07 (46)</td>
</tr>
<tr>
<td>4th instar</td>
<td>3.1 ± 0.13 (44)</td>
<td>2.3 ± 0.07 (45)</td>
</tr>
<tr>
<td>5th instar</td>
<td>3.5 ± 0.5 (2)</td>
<td>-</td>
</tr>
<tr>
<td>Prepupa</td>
<td>2.9 ± 0.09 (44)</td>
<td>2.1 ± 0.07 (39)</td>
</tr>
<tr>
<td>Pupa</td>
<td>5.6 ± 0.16 (44)</td>
<td>4.3 ± 0.08 (33)</td>
</tr>
<tr>
<td>Mean Totalb</td>
<td>26.6 ± 0.40</td>
<td>19.4 ± 0.14</td>
</tr>
<tr>
<td>DTOPc</td>
<td>4.09 ± 0.24</td>
<td>3.72 ± 0.28</td>
</tr>
</tbody>
</table>

n = 50; Numbers in parentheses are the experimental unit.

a No significant differences in the effect of temperature duration of development of life stages (\(\alpha = 0.05\)); Tukey's test.

b There were significant differences in the effect of temperature on total duration of development (\(\alpha = 0.05\)); Tukey's test.

c Days to the beginning of oviposition by the females.
turnip. Four larval instars were recorded in its life cycle with about 5% of the larvae going through a fifth instar. A previous report identified only three instars (Oliver and Chapin 1983).

Though there were no significant effects of host plant on length of development, multi-generation survivorship studies and pupal weight showed that the beetle had poor survivorship on cabbage over time. A significant amount of third generation larvae maintained on cabbage did not pupate and the few that pupated metamorphosed into adults that did not lay eggs. These results suggest that cabbage is exhibiting some degree of resistance to the beetle through the mechanism of antibiosis. Antibiosis is one of the three mechanisms of plant resistance to insects mentioned by Painter (1951). It describes the negative effects of a resistant plant on the biology of the insect attempting to use that plant as a host (Smith 1989). Antibiotic resistance by cabbage to M. ochrolouma is manifested in larval mortalities, failure of larvae to pupate and lower pupal weight. This relative poor performance of the beetle on cabbage suggests that this plant might not be suitable enough as a host to support
field populations of the beetle for many generations. This view is supported in part by a field observation made by Chamberlin and Tippins (1948) in Theodore, Alabama. They reported that a small population of the beetle was found on a large acreage of cabbage while an adjacent field with a small acreage of turnip supported a relatively high population. On collard, radish and turnip, the beetle was maintained continuously one generation after another up to four generations, and on mustard for several generations.

The poor survivorship of beetles maintained on cabbage relative to the other host plants might be due to differences in the physical and chemical parameters of the leaves of each plant. Physically, the leaves of mustard, radish and turnip appear to be similar in being of softer texture and conspicuously hirsute while the leaves of collard and cabbage are relatively tougher and glabrous. Even among these two, the leaves of cabbage are tougher than those of collard and have a waxy layer. Thus, the toughness of cabbage leaves might make them less suitable as food for M. ochroloma. Tanton (1962) suggested that the toughness of plant tissues might be related to the degree to which they can be exploited by insects. There might
also be qualitative and quantitative differences in the allelochemical constituents of each plant. Though cabbage, collard, mustard, radish and turnip belong to the family Cruciferae and are defended primarily by glucosinolates (Kjaer 1976, Feeny 1977), it has been reported that some plants in this family have evolved an additional line of defense (Usher and Feeny 1983). I speculate that cabbage probably contains one or more chemical compounds which renders it unsuitable as a host for the yellowmargined leaf beetle. An analysis of the chemical profile of the foliage of the five plants would be needed to understand the underlying mechanisms.

Similar to the situation in many insects, a 5°C increase in temperature had a significant effect on the length of development. Beetles maintained at 25°C developed significantly faster than those maintained at 20°C. However, there is the need to properly evaluate the effect of temperature at much higher and lower extremes than reported in this study. Temperature has been mentioned to be an important environmental factor affecting the life history of the beetle in the field (Oliver and Chapin 1983).
In summary, although *M. ochrolooma* completed its development from egg to adult on cabbage, collard, mustard, radish and turnip, its multi-generation survivorship was poor on cabbage. Cabbage exhibited some degree of resistance to the beetle probably through the mechanism of antibiosis resulting in larval mortalities, failure of larvae to pupate and reduced pupal weight.
CHAPTER 4

FECUNDITY AND LONGEVITY OF THE YELLOWMARGINED LEAF BEETLE
(COLEOPTERA: CHRYSOMELIDAE) ON CRUCIFERS

Introduction

Fecundity was defined by Labeyrie (1978) as the power of the females to produce functional gametes. It is an essential process in species multiplication and evolution of populations. Factors affecting fecundity in the long term affect the survival of the species. These factors include inherent capacities of the ovaries to produce a given number of eggs, the hormonal control of vitellogenesis, the environmental cues that control the timing of hormone synthesis and release and the acquisition of reserves for making the yolk (Engelmann 1984). The nature of the reproductive process in insects as exemplified by periodicity of egg maturation and oviposition suggests hormonal control (Engelmann 1968). In most insects, the corpus allatum is the source of gonadotrophic hormones important in normal oocyte development (Engelmann 1968, Chapman 1978). The neurosecretory cells of the pars intercerebralis and corpora cardiaca function as release organs of neurosecretory materials that activate the corpora allata.
The role of feeding and nutrition in insect reproduction was underscored by Johansson (1964). He mentioned that the majority of insect species will not mature eggs if they have not had an opportunity to feed. A source of glucose and essential amino acids is generally needed (de Wilde and de Loof 1973). Lack of food and reserves could affect reproduction either by impacting synthesis of the proteinaceous and lipid yolk needed for egg maturation and/or by restraining the production of hormones from the endocrine glands (Engelmann 1968). In addition to adequate nutrition, many phytophagous insects have been reported to require a specific host plant factor called "token" stimuli to stimulate oogenesis and oviposition (Matsumoto and Thorsteinson 1968, Beruter and Stadler 1971). The white cabbage butterfly, Pieris brassicae (L.), for instance, was reported to require mustard oil glucosides found in crucifers before it would lay eggs (David and Gardiner 1962).

Temperature and photoperiod are the two most important environmental factors affecting the fecundity of many insects. Since insects are poikilothersms, ambient temperature determines body temperature and this frequently
affects body size (Precht et al. 1973), and body size affects fecundity (Engelmann 1970). Temperature interacts with photoperiod to a greater or lesser extent (Masaki 1980) and this interaction has been suggested to be important in controlling the functioning of the neurosecretory cells and corpus allatum (de Wilde and de Loof 1973).

Longevity as a life history variable has been found to be directly or inversely correlated with fecundity in many insects (Rockstein and Miguel 1973). This is because the nutritional quality of food, environmental and ecological factors that greatly influence fecundity ultimately also determine how long the insect lives. Shorey (1963) reported that variations in food concentrations affected both the fecundity and longevity of the adult female cabbage looper, *Trichoplusia ni* (Hübner). Some authors have also reported an interaction between longevity, fecundity and sex in some insects. Bonjour et al. (1993) found that males of the squash bug, *Anasa tritis* (De Geer), lived longer than females probably because females required extra energy for egg production.
The fecundity and longevity of the yellowmargined leaf beetle, a pest on some cruciferous plants is not well documented. Oliver and Chapin (1983) reported that female beetles maintained on turnip foliage, on the average, laid 83 eggs and lived about 43 days. Similar information is needed on some of the other crucifer host plants. Quantifying fecundity and longevity of the beetle, in a comparative manner, on some of the crucifer host plants will reveal information about potential beetle population on field plantings of these crops.

Materials and Methods

Fecundity and longevity of a laboratory population of adult yellowmargined leaf beetles fed cabbage, collard, mustard, radish and turnip were quantified. The beetles on which fecundity and longevity data were taken were preconditioned on each host plant by continuous rearing for at least two generations in the laboratory. To begin the study, eggs were collected from female beetles that had been maintained for many generations on mustard foliage. These eggs (ca. 400) were divided into five groups and each group randomly assigned to each host plant. At eclosion, the first instars were reared on the foliage of the
assigned host plant until pupation (See Chapters 2 & 3 for rearing methods). At adult emergence, males and females were paired by host plant. Eggs were collected from these F1 adults into separate petri dishes by host plant. When the larvae eclosed, they were reared on the appropriate host foliage until pupation. As soon as the adults from this generation emerged, twenty male and twenty female, 1-day old beetles were randomly selected from each host plant colony, weighed individually and paired. Each pair of beetles was placed in a petri dish whose bottom had been lined with a single piece of filter paper. For each host, paired beetles were randomly assigned numbers from 1 to 20. The cover of each dish was labeled according to host plant and the assigned number. The dishes were arranged in plastic storage boxes (10 X 15 X 30 cm) by host plant and covered. To maintain high humidity inside each box, the bottoms were filled with water, one cm in depth, above which a piece of cardboard was placed and supported with inverted petri dish covers (5.5 cm in diameter). The boxes were arranged in an incubator (about 91 cu. cm Hotpack) maintained at a temperature of 20 ± 1°C, 50% RH and 14L:10D photophase. Paired beetles were supplied with a leaf disc
(15 cm²) of the appropriate host. Dishes were checked carefully every forty eight hours and eggs laid by females were collected and counted. During this time, a fresh leaf disc was also supplied. Beetles were maintained until either pair died. After the death of each specimen, length measurements were taken with a metric ruler. Fertility of female beetles was evaluated by monitoring percent egg hatching from the total number of eggs laid over the lifetime of five randomly selected females by host plant.

The total number of eggs laid by a female over its lifetime was recorded as its fecundity while the number of days lived by a beetle specimen from emergence till death was recorded as its longevity. Fecundity was analyzed as a one-way and longevity as a two-way analysis of variance for the effect of host plant and sex with a completely randomized design (α = 0.05 [SAS Institute 1989]). Means were separated using Tukey's studentized range test.

Morphometric data of beetles used in this study were summarized by host plant and sex (PROC MEANS [SAS Institute 1989]). The relationships of fecundity to longevity, length and weight were tested using Pearson's correlation coefficient (PROC CORR [SAS Institute 1989]). Daily
fecundity by host plant was computed by dividing the total fecundity of a female by its longevity. This variable was then analyzed as a one-way analysis of variance ($\alpha = 0.05$) for the effect of host plant.

Results

The analysis of variance test revealed a significant effect of host plant on the fecundity of *M. ochroloma* ($F = 3.90$, $df = 4$, $P = 0.0057$). The mean number of eggs per female was significantly higher for beetles maintained on turnip (490.74 ± 116.04) than for those maintained on collard (198.85 ± 28.94) (Figure 4.1). There were no significant differences in the mean number of egg per female for beetles maintained on cabbage (271.25 ± 39.11), mustard (424.95 ± 46.39), radish (440.05 ± 50.09) and turnip (Figure 4.1). The highest and lowest number of eggs laid by a single female was 1497 on turnip and 10 on collard, respectively.

Like fecundity, longevity was also significantly affected by host plant ($F = 11.40$, $df = 4$, $P = 0.0001$). There were significant differences in the longevity of beetles maintained on radish and those maintained on each of the other host plants (Figure 4.2). There were no
significant differences however, in the longevity of beetles maintained on cabbage, collard, mustard and turnip (Figure 4.2). Comparing male and female beetles, there were no significant differences in their longevities on all the host plants ($F = 0.53, \text{df} = 1, P = 0.4677$) (Figure 4.2). In addition, the interaction of plant with sex was not significant ($F = 0.51, \text{df} = 4, P = 0.7301$). The highest and lowest longevity recorded for female beetles were 186 d for a beetle maintained on radish and 22 d for a beetle maintained on turnip, respectively. While for males, the highest and lowest longevity were 186 d for a beetle maintained on radish and 16 d for a beetle maintained on collard, respectively.

Significant differences were found in daily fecundity by host plant ($F = 4.55, \text{df} = 4, P = 0.0021$). Beetles maintained on turnip and mustard had significantly higher number of eggs per female per day than beetles maintained on collard (Figure 4.3). There were no significant differences in the daily fecundity of beetles maintained on cabbage, mustard, radish and turnip (Figure 4.3). There were no significant differences in the fertility of females across host plants. The percent egg hatch were 98.7%
(940 of 952) on cabbage, 99.1% (926 of 934) on collard, 99.0% (1805 of 1823) on mustard, 99.1% (2700 of 2722) on radish and 99.0% (2401 of 2424) on turnip.

Morphometric data of beetles used in this study are summarized in Table 4.1. Female beetles were significantly heavier and longer than males. Pearson's correlation coefficients revealed that there were no significant relationships between fecundity and size (length and weight) of all beetles except those maintained on cabbage (Table 4.2). However, there were significant correlations between fecundity and longevity of beetles maintained on mustard, turnip and cabbage (Table 4.2).

Discussion

The fecundity and longevity of *M. ochroloma* on cabbage, collard, mustard and radish had not been previously reported. Oliver and Chapin (1983) had earlier reported similar information for the beetle on turnip. However, the mean fecundity and longevity recorded for females maintained on turnip in this study was higher (ca. 490 eggs and 68 days) than the mean reported by Oliver and Chapin (ca. 83 eggs and 43 days). In this study, the fecundity, longevity and daily fecundity of the
Figure 4.1. Fecundity of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip. Bars with same letter(s) are not significantly different according to Tukey's test at $\alpha = 0.05$. 
Figure 4.2. Longevity of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip. Bars with same letter(s) are not significantly different according to Tukey's test at \( \alpha = 0.05 \).
Figure 4.3. Daily fecundity of the yellowmargined leaf beetle on cabbage, collard, mustard, radish and turnip. Bars with same letter(s) are not significantly different according to Tukey's test at $\alpha = 0.05$. 
Table 4.1. Morphometric data of a laboratory population of the yellowmargin leaf beetle maintained on cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Sex</th>
<th>Length (mm) (Mean ± SE)</th>
<th>Weight (mg) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>Female</td>
<td>5.85 ± 0.09</td>
<td>11.85 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.97 ± 0.08</td>
<td>7.500 ± 0.27</td>
</tr>
<tr>
<td>Collard</td>
<td>Female</td>
<td>5.97 ± 0.09</td>
<td>11.60 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5.04 ± 0.09</td>
<td>7.400 ± 0.34</td>
</tr>
<tr>
<td>Mustard</td>
<td>Female</td>
<td>5.81 ± 0.11</td>
<td>11.55 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.83 ± 0.06</td>
<td>7.350 ± 0.22</td>
</tr>
<tr>
<td>Radish</td>
<td>Female</td>
<td>5.83 ± 0.13</td>
<td>9.789 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5.10 ± 0.14</td>
<td>6.737 ± 0.37</td>
</tr>
<tr>
<td>Turnip</td>
<td>Female</td>
<td>5.90 ± 0.11</td>
<td>10.05 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>4.89 ± 0.09</td>
<td>6.947 ± 0.25</td>
</tr>
</tbody>
</table>
Table 4.2. Pearson's correlation coefficients for the relationships of fecundity to length, weight, and longevity of a laboratory population of the yellowmargined leaf beetle maintained on cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Parameter</th>
<th>Coefficient</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>Length</td>
<td>0.4484</td>
<td>0.0474</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>0.5539</td>
<td>0.0113</td>
</tr>
<tr>
<td></td>
<td>Longevity</td>
<td>0.5794</td>
<td>0.0074</td>
</tr>
<tr>
<td>Collard</td>
<td>Length</td>
<td>0.1723</td>
<td>0.4676</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>0.1895</td>
<td>0.4236</td>
</tr>
<tr>
<td></td>
<td>Longevity</td>
<td>0.1262</td>
<td>0.5959</td>
</tr>
<tr>
<td>Mustard</td>
<td>Length</td>
<td>-0.0224</td>
<td>0.9274</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>-0.3745</td>
<td>0.1142</td>
</tr>
<tr>
<td></td>
<td>Longevity</td>
<td>0.7176</td>
<td>0.0005</td>
</tr>
<tr>
<td>Radish</td>
<td>Length</td>
<td>0.0488</td>
<td>0.8429</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>0.1250</td>
<td>0.6101</td>
</tr>
<tr>
<td></td>
<td>Longevity</td>
<td>-0.1330</td>
<td>0.5863</td>
</tr>
<tr>
<td>Turnip</td>
<td>Length</td>
<td>-0.1353</td>
<td>0.5809</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>-0.2345</td>
<td>0.3339</td>
</tr>
<tr>
<td></td>
<td>Longevity</td>
<td>0.9444</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
yellowmargined leaf beetle were quantified and compared on five cruciferous plants. Field plantings of these crops have been reported to support infestations of the beetle in many localities in the southeastern United States (Chamberlin and Tippins 1948, Rohwer et al. 1953, Anonymous 1962, Woodruff 1974, Balsbaugh 1978, Oliver and Chapin 1983). Information on fecundity and longevity are crucial to an assessment of potential beetle population on field plantings of the different crops. Results from this study showed that on the average, a female beetle maintained on turnip produced significantly higher amount of eggs than a female maintained on collard. There were no significant differences in the mean number of egg per female for beetles maintained on cabbage, mustard, radish and turnip. Relating these findings to field situations, it can be postulated that cabbage, mustard, radish and turnip each have greater potential to support a high population of the beetle than collard. Though field population densities of the beetle have never been quantified on these plants, many workers have reported field observations that indicate an unusually high number of the beetle on turnip and mustard relative to the other plants (Haeussler 1951, Rohwer et al. 1953, Oliver and Chapin 1983). In addition, Chamberlin and
Tippins (1948) observed that a small acreage of turnip plants supported a high population of the beetle in Theodore, Alabama, while an adjacent field with a larger acreage of younger tender cabbage plants supported a relatively sparse population of the beetle.

The reasons for the observed differences in the effect of host plant on the egg output of female *M. ochroloma* is not known. It is common knowledge however that insects feeding on more than one species of plant always have significantly different fecundities when maintained on each of the plants. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), for instance, was reported to have significantly different fecundity when fed on potato, *Solanum tuberosum tuberosum* L., eggplant, *S. melongena* L. and tomato, *Lycopersicon lycopersicum* (L.) (Jansson et al. 1989). These plants all belong to the family Solanaceae and are natural hosts of the beetle (Hare 1990).

Variations in the fecundity of a phytophagous insect on botanically related plants is an indication of the suitability of each plant as a host for that insect (Evans 1938). For phytophagous insects, host plant suitability is determined in part by nutritional factors contained in the plant as well as the nature of its allelochemical
constituents (Reese 1981, Janzen 1985). Nitrogen is one of the most critical nutritional factor (Brewer et al. 1985, Hare 1987, Sétamou et al. 1993). An increase in foliar nitrogen has been reported to result in increased fecundity (Hilliard and Keeley 1985, Ohmart et al. 1985). In this study, however, no significant differences were found in the amount of foliar nitrogen of the five crucifer plants on which the beetles were fed (see Chapter 2). This suggests that differences in the fecundity of *M. ochroloma* as recorded was not due to variations in the foliar nitrogen content of the crucifer plants.

With respect to allelochemicals, differences in the allelochemical constituents (Fraenkel 1959, Beck 1965) and/or the allelochemic-nutrient interactions (Reese 1983) have been cited as being of critical importance in determining the suitability of a host plant as food for phytophagous insects. Though the allelochemical profiles of the plants used in this study are not known, it is known however that cruciferous plants in general are defended by a group of chemicals collectively called glucosinolates (Kjaer 1976). These compounds are reported to be toxic to many insect species which do not normally attack crucifers (Lichtenstein et al. 1964). The yellowmargined leaf beetle
many insect species which do not normally attack crucifers (Lichtenstein et al. 1964). The yellowmargined leaf beetle as a crucifer-feeding specialist must have evolved adaptations to tolerate or detoxify these compounds. In addition to the glucosinolates, some workers have reported that some cruciferous plants have evolved a second line of defense against crucifer-adapted insects (Feeny 1977, Slansky and Feeny 1977). Such plants were reported to contain compounds such as alkaloids, curcubitacins and cardenolides. These compounds were described as atypical of plants in the family Cruciferae (Usher and Feeny 1983). Verschaffelt (1911) suggested that these compounds might account for the unsuitability of some cruciferous plants to many crucifer-feeding specialist insects. On the other hand, Reese (1983) opined that many of the deleterious physiological effects of plant allelochemics may not be due to the presence of these chemicals in the plants but to the various interactions between them and the essential nutrients. From the foregoing, it might be speculated that female beetles fed on collard foliage had relatively lower egg production than females fed on each of the other host plants because of differences in the chemistries of the plants.
There are also noticeable differences in the physical characteristics of the leaves of the plants. The leaves of mustard, radish and turnip are similar in being relatively soft and conspicuously hirsute while the leaves of cabbage and collard are relatively tough, glabrous and waxy. Since beetles fed on turnip and mustard had higher fecundities than those fed collard, it is tempting to speculate that leaf texture might be important in accounting for the variations observed in the fecundities of *M. ochroloma*. Tanton (1962) and Iheagwam (1981) mentioned that the physical toughness of plant tissues is related to the degree to which they can be exploited by phytophagous insects. Leaves of tough texture may be less suitable as food because the hardness of such leaves might physically damage the mouthparts of the insect.

In conclusion, it might be speculated that beetles fed collard had relatively lower egg production when compared to beetles fed on each of the other host plants because of differences in the physical and chemical characteristics of the foliage of the crucifer plants. Analyses of the chemical profiles of these plants would be needed to further elucidate the underlying factor(s) responsible for the variations in egg production by the beetle.
CHAPTER 5
FEEDING PREFERENCE OF THE YELLOWMARGINED LEAF BEETLE
(COLEOPTERA: CHRYsomELIDAE) AMONG CRUCIFERS

Introduction

A phytophagous insect’s host preference has been described as the predilection of the insect to select some hosts in preference to others within its host range (Beck and Schoonhoven 1980). An acceptable host is one that provides a suitable physical environment and nutritional substrate that are adequate, non-toxic and utilizable from the standpoint of digestion, assimilation and conversion into insect tissues (Beck 1974). Factors governing feeding preference could be broadly divided into two factors: botanical and ecological. Botanical factors are intrinsic to the plant and mediate interactions between the insect and the plant. Ecological factors are insect-plant interactions mediated by the plant, the insect and the environment.

Many theories have been proposed to explain insect-plant interactions as related to host plant selection and preference. The botanical instinct theory proposed by Brues (1920) suggests herbivores prefer to select and feed on plants that meet specific nutritional requirements not
offered by other plant species. Another theory, the token stimuli theory, reasoned that host preference is determined by specific secondary plant substances (Fraenkel 1959). These substances are thought to have been evolved as a means of defense to generalist herbivores. Some insects evolved mechanisms to overcome the adverse effects of these compounds and they began using them as cues to locate acceptable hosts. Kennedy (1965) proposed the dual discrimination theory which states that phytophagous insects' host preference is based on their response to both nutrient and non-nutrient constituents of the plant.

Some authors have cautioned against placing too much emphasis on the role plant chemistries play in phytophagous insects' host preference. Plant chemistry is but one of many potential factors but not the predominant determinant of host preference by phytophagous insects (Bernays and Graham 1988). The need by herbivores to avoid their natural enemies, they argued, provides the major selection pressure for a restricted diet. This was described as the concept of 'enemy free space' by Gilbert and Singer (1975) and Lawton (1978).
The feeding preference of the yellowmargined leaf beetle has never been experimentally determined. Field populations of the beetle have been recorded on turnip, collard, cabbage, mustard, radish and watercress (Chamberlin and Tippins 1948, Woodruff 1974, Oliver and Chapin 1983). These plants represent five species in three genera of the family Cruciferae. Common to these plants and the other crucifers are a group of secondary chemical compounds collectively called glucosinolates (Kjaer 1976, Feeny 1977, Usher and Feeny 1983). These compounds, while serving as a feeding deterrent to many insects (Brown 1951, Lichtenstein et al. 1964), have been reported to stimulate feeding and oviposition in the crucifer-feeding specialists (Whittaker and Feeny 1971). The yellowmargined leaf beetle as a crucifer-feeding specialist must have evolved adaptations to overcome the adverse effects of the glucosinolates.

Experiments were designed in the laboratory to study the feeding preference of larval and adult yellowmargined leaf beetles for cabbage, collard, mustard, radish and turnip foliage.
Materials and Methods

Feeding preference of first and third instars, and adult yellomargin leaf beetle for cabbage, collard, mustard, radish and turnip foliage were studied in laboratory experiments using leaf discs of each plant. For each life stage, the experiment was set up as a randomized complete block design in five circular petri dishes (140 X 15 mm). At the beginning of the study, five circular arrangements of host plants were randomly generated and these arrangements were permanently labeled A to E (Figure 5.1). Insect specimens used in these studies were from laboratory colonies and have been maintained on each plant for at least two generations. Insects from each colony were exposed to each leaf arrangement.

At each replication, leaf discs 15 cm² were cut with a cork borer from host foliage obtained from plants raised in the greenhouse and these were arranged equidistant from each other in a circular fashion on top of moistened filter paper in the petri dishes. The pattern of arrangement is as shown in Figure 5.1. For each life stage and at each replication, twenty 1-day old specimens were obtained from each of the five laboratory colonies of each host plant and they were exposed to the leaf arrangement according to the
scheme outlined in Table 5.1. Insect specimens were placed at about the center of each dish. The dishes were placed on trays and the trays were arranged on benches in a growth room maintained at 20°C, 70% RH and 14:10(L:D) hours photoperiod. The experiment was replicated five times at weekly intervals. The sequence of replication of the experiment is shown in Table 5.1. For each life stage, replication over time was the block and the five leaf arrangements were replicates in each block. Feeding preference of first instars was measured as the number of larvae found feeding on each leaf disc after about 24 h. The preference of third instars was measured by both the number of larvae found feeding on each leaf disc as well as the amount of foliage consumed after about 24 h. Adult feeding preference was quantified as the amount of foliage consumed after about 24 h. The amount of foliage of each plant consumed was quantified by weighing (to the nearest mg) each leaf disc before and after insects were allowed to feed on them. The difference between the initial and final weight of each leaf disc was the amount of foliage consumed. Five leaf discs of each host plant to which beetles were not exposed were set up as control in petri dishes.
Figure 5.1. Five leaf arrangements of crucifer plants used in the feeding preference studies of yellowmargined leaf beetle for the foliage of cabbage, collard, mustard, radish and turnip. (CB = Cabbage, CL = Collard, M = Mustard, R = Radish, T = Turnip).
Table 5.1. Sequence of replication of the feeding preference studies of the yellowmargined leaf beetle for the foliage of cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>LDA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cabbage</td>
<td>Mustard</td>
<td>Radish</td>
<td>Collard</td>
<td>Turnip</td>
</tr>
<tr>
<td>B</td>
<td>Collard</td>
<td>Turnip</td>
<td>Cabbage</td>
<td>Mustard</td>
<td>Radish</td>
</tr>
<tr>
<td>C</td>
<td>Mustard</td>
<td>Radish</td>
<td>Collard</td>
<td>Turnip</td>
<td>Cabbage</td>
</tr>
<tr>
<td>D</td>
<td>Radish</td>
<td>Collard</td>
<td>Turnip</td>
<td>Cabbage</td>
<td>Mustard</td>
</tr>
<tr>
<td>E</td>
<td>Turnip</td>
<td>Cabbage</td>
<td>Mustard</td>
<td>Radish</td>
<td>Collard</td>
</tr>
</tbody>
</table>

<sup>a</sup> At first replication, beetles from cabbage colony were exposed to leaf disc arrangement ‘A’, beetles from collard colony to arrangement ‘B’, etc. At the second replication, beetles from radish colony were exposed to arrangement ‘C’, beetles from collard colony to arrangement ‘D’, etc. At the fifth replication, beetles from turnip colony were exposed to arrangement ‘A’, beetles from radish colony to arrangement ‘B’, beetles from collard colony to arrangement ‘E’, etc.

<sup>b</sup> Leaf disc arrangement as per Figure 5.1.
Preference for each plant was determined by comparing the mean number of larvae associated with the leaf disc of each plant (first and third instar larvae) and the mean foliage consumption (third instar larvae and adult) after about 24 h. Data were analyzed separately for each life stage as a randomized block design (PROC GLM [SAS Institute 1989]). For the third larval instar, the number of larvae associated with leaf disc of each plant was correlated with leaf consumption (PROC CORR [SAS Institute 1989]). A multivariate analysis was also carried out to test whether the host plant on which beetles were reared had any influence on their feeding preferences.

Results

Each of the three life stages of the yellowmargined leaf beetle examined in this study showed strong feeding preference for some crucifer plants over the others. There was a significant difference in the number of first instar larvae associated with the foliage of the different crucifer plants ($F = 44.22, df = 4, P = 0.0001$). The third instar larvae also showed strong feeding preference as determined by number of larvae associated with the foliage of each plant ($F = 9.76, df = 4, P = 0.0003$) and the amount
of foliage consumed ($F = 15.23, \text{df} = 4, \ P = 0.0001$).

Feeding preference of adult beetles was also significantly different ($F = 4.90, \text{df} = 4, \ P = 0.0090$).

First instar yellowmargined leaf beetle showed strong feeding preference for turnip foliage and least preference for cabbage, collard and radish (Table 5.2). The feeding preferences of third instars and adult beetles were similar. Both consumed a significantly higher amount of each of turnip and mustard foliage over those of collard and cabbage (Table 5.3). For the third instar, there was a positive and significant correlation ($r = 0.98, \ P = 0.0035$) between the number of third instars associated with the foliage of each plant and the amount of foliage consumed.

In general, turnip and mustard were the preferred hosts, followed by radish, collard and cabbage in that order.

Results of multivariate analysis revealed that the host plant on which beetles were reared did not have any influence on their feeding preferences. The Wilk’s Lambda statistics and their respective p-values were 0.4048 and 0.3520 for first instar larvae, 0.4394 and 0.7463 for third instar larvae and 0.4696 and 0.8170 for adult beetles.
Table 5.2. Feeding preference of first and third instar larval yellowmargined leaf beetle as determined by number of larvae feeding on the foliage of cabbage, collard, mustard, radish and turnip.

<table>
<thead>
<tr>
<th>Host plant</th>
<th>First instar (Mean ± SE)</th>
<th>Third instar (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>1.12 ± 0.26c</td>
<td>1.88 ± 0.31c</td>
</tr>
<tr>
<td>Collard</td>
<td>1.52 ± 0.34c</td>
<td>2.32 ± 0.44c</td>
</tr>
<tr>
<td>Mustard</td>
<td>5.96 ± 1.07b</td>
<td>4.80 ± 0.49b</td>
</tr>
<tr>
<td>Radish</td>
<td>2.32 ± 0.42c</td>
<td>3.36 ± 0.58bc</td>
</tr>
<tr>
<td>Turnip</td>
<td>9.16 ± 1.05a</td>
<td>7.04 ± 0.85a</td>
</tr>
</tbody>
</table>

Means within columns followed by same letter(s) are not significantly different (α = 0.05, Tukey's test [SAS Institute 1989]). For each column, df = 4.
Table 5.3. Feeding preference of third instar larvae and adult yellowmargined leaf beetle for crucifer plants as determined by leaf consumption (mg).

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Third instar (Mean ± SE)</th>
<th>Adult (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>16.16 ± 2.21c</td>
<td>24.15 ± 3.52c</td>
</tr>
<tr>
<td>Collard</td>
<td>19.84 ± 2.65c</td>
<td>36.19 ± 5.36bc</td>
</tr>
<tr>
<td>Mustard</td>
<td>45.55 ± 2.95a</td>
<td>50.04 ± 2.63a</td>
</tr>
<tr>
<td>Radish</td>
<td>32.61 ± 2.77b</td>
<td>43.89 ± 4.05ab</td>
</tr>
<tr>
<td>Turnip</td>
<td>55.51 ± 3.61a</td>
<td>49.68 ± 3.21a</td>
</tr>
</tbody>
</table>

Means within columns followed by same letter(s) are not significantly different ($\alpha = 0.05$, Tukey’s test [SAS Institute 1989]). For each column, df = 4.
Discussion

The feeding preference of larval and adult yellowmargined leaf beetle for the foliage of cabbage, collard, mustard, radish and turnip were investigated in laboratory experiments. Several authors have reported this beetle to be associated with field plantings of these crops (Chamberlin and Tippins 1948, Jolivet 1950, Rohwer et al. 1953, Woodruff 1974, Oliver and Chapin 1983). Though the feeding preference of this beetle has never been experimentally determined, many authors have speculated that it seems to prefer turnip (Chamberlin and Tippins 1948, Oliver and Chapin 1983). Results from the present study confirm this speculation, and in addition, the beetle was found to show strong feeding preference for mustard as well. Thus, in a choice situation, larval and adult yellowmargined leaf beetles prefer to feed on the foliage of turnip and mustard. Collard and cabbage foliage were not preferred. Preference for radish was between these extremes. These results suggest field plantings of turnip and mustard will be more susceptible to beetle infestations than those of collard and cabbage. At present, there are no quantitative data on the relative populations of the beetle on field plantings of any of these crops.
Nevertheless, many authors have observed and reported that field plantings of turnip and mustard supported a relatively high population of the beetle in several locations in the US (Haeussler 1951, Oliver 1956, Spink 1959, Anonymous 1960, Anonymous 1976). In addition, Chamberlin and Tippins (1948) reported that a large acreage of young tender cabbage plants supported a sparse population of the beetle while an adjacent field with a small acreage of turnip supported a relatively higher population at Theodore, Alabama.

In general, it is common for phytophagous insects feeding on many plants, even on plants within the same family, to have strong feeding preference for some plant species over the others. Palaniswamy and Lamb (1992) reported that two flea beetle species, Phyllotreta cruciferae (Goeze) and P. striolata (F.), both crucifer-feeding specialists, when given a choice among plants within their host range, prefer to feed on Brassica species rather than Sinapis species. The feeding preferences of these beetles were examined on seven species of crucifers, Brassica oleracea L., B. napus L., B. campestris L., B. juncea (L.), B. nigra (L.) Koch, Sinapis alba L. and
S. arvensis L. Preference was measured as the number of insects associated with the foliage of each plant and the severity of damage.

The reasons for the preference of the yellowmargined leaf beetle for turnip and mustard foliage over those of collard and cabbage are not known. It is known, however, that many phytophagous insects discriminate among plants within their host range based on nutrient and allelochemical contents. Kjaer (1960) demonstrated that the flea beetle, P. cruciferae, responds selectively to different crucifer plants based on their nutrient and allelochemical contents. In the present study, the nutrient and allelochemical profiles of the plants used in this study are not completely known. Analysis of the nitrogen content of the foliage of each plant revealed no significant differences (See Chapter 2). With respect to the allelochemicals, several authors have reported that these compounds serve as either attractants, feeding stimulants or as feeding deterrents to many phytophagous insects (Hicks 1974, Schoonhoven 1982, Andersen and Metcalf 1986). In the crucifers, allyl isothiocyanate, a compound produced from an enzymatic hydrolysis of glucosinolates,
has been reported to serve as a feeding attractant to many crucifer-feeding beetles (Burgess and Wiens 1980). In addition to this compound, some authors have also reported that some cruciferous plants contain certain other compounds that are feeding deterrents, especially to many chrysomelid beetles (Matsuda 1976, Nielsen et al. 1977, Nielsen 1978). These compounds were identified as cucurbitacins, cardenolides, alkaloids and flavonoids (Usher and Feeny 1983, Matsuda 1976). Since the allelochemical profiles of the plants used in this study have not yet been determined, it might not be possible to determine whether the plants on which the yellowmargin leaf beetle preferred to feed contain attractants and/or feeding stimulants that are absent in the non-preferred plants; or that the preferred plants were lacking in antifeedant chemistries which may be present in the non-preferred plants. A study of the allelochemical profiles of each of these plants will be needed to understand if the feeding preference of the beetle is due to plant chemistry.

The physical characteristics of the foliage of the plants used in this study seem to correlate with the
feeding preference of the beetle. The preferred plants, turnip and mustard, have relatively soft foliage while the foliage of collard and cabbage, which were less preferred, are relatively tough and waxy. On the basis of this observation, it might be speculated that preference of the yellowmargined leaf beetle for the foliage of turnip and mustard over those of collard and cabbage was due in part to differences in leaf texture of the plants. Tanton (1962) and Iheagwam (1981) mentioned that the physical toughness of plant tissues is related to the degree to which they can be exploited by phytophagous insects. Leaves of tough texture may be less suitable as food because the hardness of such leaves might physically damage the mouthparts of the insect.
CHAPTER 6

SPATIAL DISPERSION OF THE YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) ON MUSTARD.

Introduction

Spatial dispersion is one of the most characteristic ecological properties of a species (Taylor 1984). In nature, spatial dispersion of animals and plants corresponds broadly into one of three patterns: regular, in which individuals are spaced evenly at regular intervals; random, in which the occurrence of each individual is independent of any other and clumped, in which the presence of an individual increases the likelihood of another being found nearby.

Mathematical distribution models are used to describe spatial patterns. In general, these models relate the variance of counts to the mean. For random spatial patterns, the variance equals the mean and this pattern is described by the Poisson statistical distribution. In nature, populations are rarely found to be randomly dispersed, and the probability of finding an individual in a unit area can rarely be predicted by the Poisson distribution (Poole 1974). Aggregated spatial patterns are
characterized by the variance of counts exceeding the mean. Mathematical distribution models used to describe aggregated spatial patterns include the Neyman type A, negative binomial, Poisson with added zeros, positive binomial, Logarithmic with added zeros etc. In entomology, the most frequently used aggregated distribution model is the negative binomial (Ruesink 1980). The negative binomial is described by two parameters, the mean $\bar{x}$ and the exponent $k$, which is a measure of the degree of clumping, and is often referred to as the dispersion parameter (Southwood 1978). Values of $k$ range from between zero and infinity. A small $k$ (between zero and 8) implies that the pattern is aggregated, and the smaller the $k$, the greater the aggregation (Poole 1974). Values of $k$ increasing beyond eight till infinity suggest that the spatial pattern is random, and the negative binomial simplifies to the Poisson. The usefulness of $k$ as an index of dispersion has however been reported to be limited because it has been found that $k$ values are not constant for a population but often increases with the mean (Anscombe 1949, Bliss and Owen 1958, Taylor et al. 1978).
For practical purposes, it is often necessary to determine whether the Poisson or the negative binomial distribution model adequately describes a frequency distribution of counts. Both of these models rely on the relationship between the variance and the mean of counts. Taylor (1961, 1965, 1971) showed that this relationship obeys a power law which holds in a series of distribution from regular through random to highly aggregated patterns. Taylor's power law is expressed as:

$$s^2 = ax^b$$

where $a$ and $b$ are constants. The coefficient $a$ varies with the sampling technique and habitat (Southwood 1978) while the exponent $b$ is the index of aggregation, characteristic of the species (Taylor et al. 1978). Mathematically, the two coefficients are estimated from the power law equation by a linear regression of $\log s^2$ on $\log x$. Values of $b$ range from 0 when the distribution is regular through 1, when the distribution is random to infinity when there is maximum aggregation (Taylor 1961).

Another index of aggregation that has proved useful in spatial pattern studies is the Iwao patchiness regression
(Iwao 1968). Similar to Taylor's power law, this procedure also uses the variance-mean relationship to generate an index of aggregation. Iwao's patchiness regression relates mean crowding, \( m = \bar{x} + \left[ s^2/\bar{x} - 1 \right] \) (Lloyd 1967) to the mean \( \bar{x} \) using the linear regression, \( m = \alpha + \beta \bar{x} \). The intercept \( \alpha \) is an index of basic contagion, and the slope \( \beta \) is related to the pattern in which the organism utilizes its habitat (Iwao 1970). Distribution patterns may be regular (\( \beta < 1 \)), random (\( \beta = 1 \)) or with increasing aggregation (\( \beta > 1 \)).

In this chapter, the objective was to determine spatial dispersion patterns of yellowmargined leaf beetle immatures and adults on field planted mustard.

**Materials and Methods**

Sampling for spatial dispersion of immature and adult yellowmargined leaf beetle was carried out on three plots of mustard plant (cv 'Florida broadleaf') located at research stations of the Louisiana State University Agricultural Experiment Station. The first two plots (designated plots one and two, respectively) were located at the St. Gabriel Research Station, Iberville Parish,
Louisiana and were sampled in the fall of 1992 through the spring of 1993. The third plot, plot three, was located at the Burden Research Station, East Baton Rouge Parish, Louisiana and was sampled in the fall of 1995. In all cases, the sampling scheme was stratified random sampling, in which plots were divided into four equal quadrants and equal number of plants was randomly sampled from each quadrant.

The first plot, plot one, was planted with mustard on August 1, 1992. The plot consisted of four rows of mustard, each row about 20 meters long. The plot was sampled five times during the fall of 1992. The sampling dates were September 23, October 9, 16, 23, and 30, 1992. Eight plants/quadrant were sampled on the first sampling date. During subsequent samplings, this number was reduced to five. Planting on plot two was done on September 25, 1992. There were eight rows of mustard plant, each row about 22 meters long. This plot was sampled three times, November 13, 1992, January 18 and 25, 1993. Eight plants/quadrant were sampled on each sampling date. In both plots, the plants were 'destructively' sampled, that is, the stem of a sampled plant was cut with a pair of
shears just above the soil surface. Thereafter, the plant was sectioned into three equal parts designated 'upper', 'middle' and 'lower' sections. The upper section was the top one-third, the lower, the bottom one-third, and the 'middle' section was between the upper and lower sections. Each plant section was placed individually in a plastic bag (20.32 by 40.64 cm), labeled accordingly and transported to the laboratory. In the laboratory, plant sections including the inside of respective bags were carefully inspected and the number of beetle specimens were counted and recorded by life stage. The third plot was planted with mustard on August 22, 1995. This plot consisted of four rows of mustard, each row about 40 meters long. The plot was sampled five times at weekly intervals. The sampling dates were November 8, 15, 22, 29 and December 7, 1995. Like the St. Gabriel plots, plants were destructively sampled but were not sectioned into three parts. The sampling unit in this case was a whole mustard plant rather than plant sections. Twelve plants/quadrant were randomly selected from each of the four quadrants. Plants were transported to the laboratory individually in plastic bags (30.48 by 50.8 cm). In the laboratory, the plants including the inside of respective bags were
carefully inspected and the number of yellowmargined leaf beetle life stages were counted and recorded.

For the purpose of statistical analysis, larvae were grouped by size into 'small' larvae (instars 1 and 2) and 'large' larvae (instars 3, 4 and the prepupa). Count data for the life stages from the plots at the St. Gabriel Research Station were analyzed separately for both within- and between-plant distribution as an analysis of variance (PROC GLM [SAS Institute 1989]). Count data from the plot at Burden Research Station were analyzed for between-plant distribution only as analysis of variance in SAS. In addition, means and variances of counts per plant were calculated for each plot on each sampling date by life stages. Spatial dispersion indices were calculated separately for each plot using Taylor's power law (Taylor 1961) and Iwao's patchiness regression (Iwao 1968). The general linear model of SAS was used to estimate the linear regressions (PROC GLM [SAS Institute 1989]). Student's t-tests were used to determine if the slopes of the regression lines were significantly greater than 1 at \( \alpha = 0.05 \). Count data were also fit to the negative binomial distribution using the BestFit program (BestFit 1993). Only count data from the Burden Research
Station plot were fit into the negative binomial distribution. The population of beetles at the St. Gabriel Research Station plots was low. The count for each life stage was combined over all sampling dates for the fit. The chi-square goodness of fit values were compared to tabular values at k-1 degrees of freedom. Estimates of the values of k for the negative binomial distribution were computed for each life stage by sampling date using the methods of Bliss and Fisher (1953).

**Results**

There were no significant differences in the within-plant counts of beetles on both plots at the St. Gabriel Research Station (Table 6.1). This implies that beetles were randomly distributed on each mustard plant. There were however significant differences in the between-plant counts of small larvae ($F = 5.80$, $df = 3$, $P = 0.0007$) and large larvae ($F = 8.77$, $df = 3$, $P = 0.0001$) in the first plot (Table 6.2). There were no significant differences in the counts of egg masses, pupae and adults in this plot. In the second plot, significant differences were found in the between-plant counts of adult beetles only ($F = 5.89$, $df = 3$, $P = 0.0007$). Since no significant
differences were found in the within-plant counts of beetles in the earlier samplings, subsequent sampling at the Burden Research station plot was done by taking a whole plant as a sampling unit rather than plant sections. At the Burden plot, significant differences were found in the between-plant counts of egg masses ($F = 5.82, \text{df} = 3, P = 0.0008$), small larvae ($F = 3.16, \text{df} = 3, P = 0.0254$) and large larvae ($F = 5.79, \text{df} = 3, P = 0.0008$) (Table 6.2). There were no significant differences in the between-plant counts of pupae and adults. In general, more beetles were found in quadrants one and two, where beetle infestations were first recorded earlier in the season than in the rest of the plots.

With respect to fit of the data to Taylor’s power law and Iwao’s patchiness regression, all count data from the three plots yielded significant regression lines and provided good fit. Estimates of the indices of aggregation and coefficients of determination ($R^2$) for the three sites are shown in Tables 6.3 to 6.5. Slopes from both regression models were significantly different from 1 ($\alpha = 0.05$), indicating an aggregated spatial distribution.
Data of counts for the different life stages of the beetle from the plot at the Burden Research Station were not described by the negative binomial distribution, except the count of 'large' larvae (Table 6.6). Nevertheless, estimates of k values, the index of aggregation, were computed for all the life stages (Table 6.7). In general, k values indicated an aggregated spatial pattern for all life stages except the 'small' larvae.

Discussion

The results of Taylor's power law, Iwao's patchiness regression and estimates of k from the negative binomial distribution model suggest an aggregated spatial pattern for populations of yellowmargined leaf beetle on mustard at the three plots sampled. Aggregated spatial distribution is probably dictated by the behavior of the adult beetles feeding close together. Female beetles lay eggs indiscriminately as they feed and wander around on the host lamina. This behavior ensures that egg masses are laid in close proximities. In the laboratory, it has been observed that the first and second instar larvae always congregate and feed together on the host foliage. In the later instars, the larvae tend to move around and at the time of
Table 6.1. Within-plant count of the distribution of yellowmargined leaf beetle life stages on mustard at the St. Gabriel Research Station, 8/92 to 1/93.

<table>
<thead>
<tr>
<th>Plant Section</th>
<th>Counts of life stages (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg 'Small' Larvae 'Large' Larvae Pupa Adult</td>
</tr>
<tr>
<td><strong>Plot One</strong></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>0.11 ± 0.46 ± 0.32 ± 0.02 ± 0.04 ±</td>
</tr>
<tr>
<td></td>
<td>0.09a 0.13a 0.08a 0.01a 0.03a</td>
</tr>
<tr>
<td>Middle</td>
<td>0.04 ± 0.52 ± 0.32 ± 0.01 ± 0.04 ±</td>
</tr>
<tr>
<td></td>
<td>0.03a 0.12a 0.09a 0.01a 0.02a</td>
</tr>
<tr>
<td>Lower</td>
<td>0.02 ± 0.46 ± 0.32 ± 0.04 ± 0.04 ±</td>
</tr>
<tr>
<td></td>
<td>0.01a 0.13a 0.09a 0.03a 0.02a</td>
</tr>
<tr>
<td><strong>Plot Two</strong></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>0.11 ± 0.26 ± 0.04 ± 0a 0.05 ±</td>
</tr>
<tr>
<td></td>
<td>0.11a 0.07a 0.02a 0.02a</td>
</tr>
<tr>
<td>Middle</td>
<td>0.05 ± 0.09 ± 0.02 ± 0a 0.05 ±</td>
</tr>
<tr>
<td></td>
<td>0.05a 0.04a 0.01a 0.02a</td>
</tr>
<tr>
<td>Lower</td>
<td>0.04 ± 0.13 ± 0.05 ± 0a 0.05 ±</td>
</tr>
<tr>
<td></td>
<td>0.04a 0.06a 0.03a 0.02a</td>
</tr>
</tbody>
</table>

Means within columns followed by same letters are not significantly different according to Tukey's test at \( \alpha = 0.05 \).
Table 6.2. Between-plant count of the distribution of yellowmargined leaf beetle life stages on three plots of mustard at the Louisiana Agricultural Experiment Stations.

<table>
<thead>
<tr>
<th>Quad</th>
<th>Egg</th>
<th>'Small' Larvae</th>
<th>'Large' Larvae</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>St. Gabriel, Plot One</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>0.18 ± 0.14a</td>
<td>1.01 ± 0.22a</td>
<td>0.73 ± 0.16a</td>
<td>0.04 ± 0.02a</td>
<td>0.09 ± 0.05a</td>
</tr>
<tr>
<td>Q2</td>
<td>0.02 ± 0.02a</td>
<td>0.41 ± 0.12b</td>
<td>0.33 ± 0.09b</td>
<td>0.05 ± 0.04a</td>
<td>0.04 ± 0.02a</td>
</tr>
<tr>
<td>Q3</td>
<td>0a</td>
<td>0.20 ± 0.09b</td>
<td>0.11 ± 0.04b</td>
<td>0.04 ± 0.03a</td>
<td>0.01 ± 0.01a</td>
</tr>
<tr>
<td>Q4</td>
<td>0.01 ± 0.01a</td>
<td>0.37 ± 0.12b</td>
<td>0.12 ± 0.05b</td>
<td>0a</td>
<td>0.01 ± 0.01a</td>
</tr>
<tr>
<td><strong>St. Gabriel, Plot Two</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>0.19 ± 0.15a</td>
<td>0.18 ± 0.06a</td>
<td>0.07 ± 0.03a</td>
<td>0a</td>
<td>0.06 ± 0.03a</td>
</tr>
<tr>
<td>Q2</td>
<td>0.07 ± 0.07a</td>
<td>0.29 ± 0.10a</td>
<td>0.04 ± 0.04a</td>
<td>0a</td>
<td>0.05 ± 0.02a</td>
</tr>
<tr>
<td>Q3</td>
<td>0a</td>
<td>0.08 ± 0.04a</td>
<td>0.04 ± 0.02a</td>
<td>0a</td>
<td>0.01 ± 0.01b</td>
</tr>
<tr>
<td>Q4</td>
<td>0a</td>
<td>0.08 ± 0.03a</td>
<td>0a</td>
<td>0a</td>
<td>0.01 ± 0.01b</td>
</tr>
</tbody>
</table>

Table 6.2 (continued)
Counts of life stages (Mean ± SE)

<table>
<thead>
<tr>
<th>Quad</th>
<th>Egg</th>
<th>'Small' Larvae</th>
<th>'Large' Larvae</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burden Research Station Plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>7.13 ± 1.58ab</td>
<td>12.2 ± 0.12a</td>
<td>16.1 ± 3.06a</td>
<td>1.98 ± 1.04a</td>
<td>4.63 ± 1.25a</td>
</tr>
<tr>
<td>Q2</td>
<td>12.4 ± 2.79a</td>
<td>12.8 ± 0.33a</td>
<td>9.33 ± 1.17b</td>
<td>0.97 ± 0.45a</td>
<td>3.08 ± 0.56a</td>
</tr>
<tr>
<td>Q3</td>
<td>3.06 ± 2.79b</td>
<td>12.1 ± 0.05a</td>
<td>6.70 ± 0.89b</td>
<td>0.45 ± 0.37a</td>
<td>2.28 ± 0.41a</td>
</tr>
<tr>
<td>Q4</td>
<td>4.33 ± 1.32b</td>
<td>12.4 ± 0.15a</td>
<td>7.97 ± 1.25b</td>
<td>0.37 ± 0.11a</td>
<td>2.42 ± 0.48a</td>
</tr>
</tbody>
</table>

For each site, means within columns followed by same letter(s) are not significantly different according to Tukey's test at α = 0.05. Q1 to Q4 = Quadrants 1 to 4.
Table 6.3. Taylor’s power law and Iwao’s patchiness regression statistics for yellowmargined leaf beetle life stages taken from mustard on plot one at the St. Gabriel Research Station, Iberville Parish, LA, in the fall of 1992.

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Egg</th>
<th>'Small'</th>
<th>'Large'</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taylor’s power law</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log a ± SE</td>
<td>-0.02 ± 0.009</td>
<td>-0.145 ± 0.05</td>
<td>-40.9 ± 2.2</td>
<td>-0.004 ± 0.01</td>
<td>-0.006 ± 0.005</td>
</tr>
<tr>
<td>b ± SE</td>
<td>2.64 ± 0.06</td>
<td>2.05 ± 0.11</td>
<td>15.1 ± 0.79</td>
<td>1.73 ± 0.09</td>
<td>1.49 ± 0.05</td>
</tr>
<tr>
<td>R²</td>
<td>0.97</td>
<td>0.86</td>
<td>0.86</td>
<td>0.86</td>
<td>0.95</td>
</tr>
</tbody>
</table>

| **Iwao’s patchiness regression** |     |         |         |      |       |
| a ± SE                 | -3.06 ± 0.079 | -1.75 ± 0.18 | -2.41 ± 0.09 | -0.83 ± 0.09 | -0.507 ± 0.04 |
| b ± SE                 | 4.02 ± 0.07 | 2.46 ± 0.11 | 1.09 ± 0.01 | 1.83 ± 0.09 | 1.50 ± 0.04 |
| R²                     | 0.98 | 0.88 | 0.99 | 0.85 | 0.96 |
Table 6.4. Taylor’s power law and Iwao’s patchiness regression statistics for yellowmargined leaf beetle life stages taken from mustard on plot two at the St. Gabriel Research Station, Iberville Parish, LA, in the spring of 1993.

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Egg</th>
<th>'Small' Larvae</th>
<th>'Large' Larvae</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taylor’s power law</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log a ± SE</td>
<td>-0.003 ± 0.009</td>
<td>-0.03 ± 0.02</td>
<td>-0.01 ± 0.012</td>
<td>-</td>
<td>-0.001 ± 0.001</td>
</tr>
<tr>
<td>b ± SE</td>
<td>3.07 ± 0.03</td>
<td>1.74 ± 0.13</td>
<td>1.45 ± 0.13</td>
<td>-</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>R²</td>
<td>0.99</td>
<td>0.84</td>
<td>0.78</td>
<td>-</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Iwao’s patchiness regression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α ± SE</td>
<td>-3.54 ± 0.015</td>
<td>-0.78 ± 0.16</td>
<td>-0.55 ± 0.15</td>
<td>-</td>
<td>-0.087 ± 0.01</td>
</tr>
<tr>
<td>β ± SE</td>
<td>4.52 ± 0.14</td>
<td>1.76 ± 0.14</td>
<td>1.54 ± 0.01</td>
<td>-</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>R²</td>
<td>0.97</td>
<td>0.83</td>
<td>0.78</td>
<td>-</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Table 6.5. Taylor’s power law and Iwao’s patchiness regression statistics for yellowmargined leaf beetle life stages taken from mustard plot at the Burden Research Station, East Baton Rouge Parish, LA, in the fall of 1995.

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Egg</th>
<th>'Small' Larvae</th>
<th>'Large' Larvae</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taylor’s power law</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log $a \pm SE$</td>
<td>-0.68 ± 0.27</td>
<td>-54.39 ± 3.38</td>
<td>-0.74 ± 0.59</td>
<td>-0.25 ± 0.12</td>
<td>-0.33 ± 0.15</td>
</tr>
<tr>
<td>$b \pm SE$</td>
<td>1.95 ± 0.14</td>
<td>21.2 ± 1.31</td>
<td>2.15 ± 0.26</td>
<td>2.96 ± 0.16</td>
<td>2.04 ± 0.11</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.91</td>
<td>0.94</td>
<td>0.79</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td><strong>Iwao’s patchiness regression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha \pm SE$</td>
<td>5.42 ± 2.67</td>
<td>-7.41 ± 0.07</td>
<td>-7.88 ± 3.92</td>
<td>-9.19 ± 1.79</td>
<td>-1.40 ± 0.35</td>
</tr>
<tr>
<td>$\beta \pm SE$</td>
<td>2.07 ± 0.25</td>
<td>1.49 ± 0.05</td>
<td>2.58 ± 0.31</td>
<td>7.87 ± 0.76</td>
<td>1.90 ± 0.07</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.79</td>
<td>0.97</td>
<td>0.79</td>
<td>0.86</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Table 6.6. Chi-square goodness of fit statistics for the negative binomial distribution of yellowmargined leaf beetle life stages collected from mustard at the Burden Research Station, fall 1995.

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Mean # of specimens collected</th>
<th>df</th>
<th>chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>6.662</td>
<td>104</td>
<td>299.96</td>
</tr>
<tr>
<td>'Small' larvae</td>
<td>2.304</td>
<td>40</td>
<td>107.04</td>
</tr>
<tr>
<td>'Large' larvae</td>
<td>11.029</td>
<td>132</td>
<td>23.00*</td>
</tr>
<tr>
<td>Pupa</td>
<td>0.9417</td>
<td>60</td>
<td>2.4 X 10^14</td>
</tr>
<tr>
<td>Adult</td>
<td>3.1041</td>
<td>68</td>
<td>12584.86</td>
</tr>
</tbody>
</table>

* indicates distribution did not differ significantly from a negative binomial distribution at $\alpha = 0.05$. 
Table 6.7. Estimates of the values of k for the negative binomial distribution of yellowmargined leaf beetle life stages taken from mustard at the Burden Research Station, East Baton Rouge Parish, LA, in the fall of 1995.

<table>
<thead>
<tr>
<th>Date</th>
<th>Egg</th>
<th>'Small' larvae</th>
<th>'Large' larvae</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 November</td>
<td>0.2209</td>
<td>-30.55</td>
<td>1.530</td>
<td>0.1423</td>
<td>0.3281</td>
</tr>
<tr>
<td>15 November</td>
<td>0.2057</td>
<td>-14.33</td>
<td>0.6962</td>
<td>0.0352</td>
<td>0.6429</td>
</tr>
<tr>
<td>22 November</td>
<td>0.0699</td>
<td>-12.29</td>
<td>0.3703</td>
<td>0.3176</td>
<td>1.1894</td>
</tr>
<tr>
<td>29 November</td>
<td>0.3196</td>
<td>-13.66</td>
<td>0.5987</td>
<td>0.1519</td>
<td>1.1153</td>
</tr>
<tr>
<td>7 December</td>
<td>0.6516</td>
<td>-12.04</td>
<td>0.5209</td>
<td>0.1082</td>
<td>0.7329</td>
</tr>
</tbody>
</table>
pupation, the mature larvae tend to come together and spin their cocoons in close proximities. This will ensure that emerging adults will also be aggregated. In the field plots, more beetles were found throughout the sampling period at the point where infestations were first noticed. As the population grows, infestation spreads round the whole field.

Aggregated behavior by the yellowmargined leaf beetle might be a reaction to injury-related release of a volatile mustard oil, allyl isothiocyanate, one of several molecules produced from an enzymatic hydrolysis of glucosinolates. The enzyme and its substrates are compartmentalized so that reaction occurs only when the plant is injured (Feeny et al. 1970, Vincent and Stewart 1984). This phenomenon has been observed in the flea beetle, *Phyllotreta cruciferae* (Goeze) (Vaughn and Hoy 1993).

A knowledge of the distribution of counts of an insect pest is an essential element for the development of a sequential sampling plan (Peng and Brewer 1994). The development of a sequential sampling plan is of particular value for the assessment of pest density in relation to control measures when pest density has reached a certain
level (Southwood 1978). No attempts were made to use the results from the present studies to develop a sequential sampling plan for the yellowmargined leaf beetle on mustard. In order to do this, data are needed for at least two planting seasons, preferably on the same field having a substantial beetle infestation. The St. Gabriel plots had low beetle infestations probably due to a history of pesticide usage. The Burden plot had high beetle infestations but the beetle arrived late on the plants resulting in an asynchronous relationship between growing beetle population and plants age. Overall, results from these studies provided preliminary insight into spatial distribution and field ecology of the yellowmargined leaf beetle.
CHAPTER 7

INSECTICIDE SUSCEPTIBILITY AND DETOXIFYING ENZYMES OF THE
YELLOWMARGINED LEAF BEETLE (COLEOPTERA: CHRYSONEMELIDAEE)

Introduction

Insecticide susceptibility can be affected by factors such as the metabolic rate of the insect (O’Brien 1967), its diet (Ghidiu et al. 1990, Moldenke et al. 1992), the efficacy of cellular detoxicating enzymes (Matsumura 1985) and environmental factors such as pH, humidity and temperature (Hadaway and Barlow 1957, Fisher 1991). Variations in insecticide susceptibility of many insects are due in part to the fact that the different insecticides have different modes of action and act at different target sites. The organophosphate and carbamate insecticides inhibit cholinesterase activities leading to disruption of synaptic functions (O’Brien 1967, Baillie 1985). The pyrethroids and the organochlorines disrupt the functioning of the sodium ion channels (Sattelle and Yamamoto 1988).

The extensive use of chemical pesticides has resulted in serious ecological and environmental problems (Hagen and Franz 1973). Smith (1970) listed some of the problems associated with pesticide use as outbreaks of secondary
pests, adverse effects on non-target organisms, objectionable pesticide residues, direct hazards to the user, and pest resistance. Cremlyn (1978) defined insecticide resistance as the selection of insect strains tolerant to doses of insecticide poison that would kill the majority of individuals in the normal population. Insect resistance to insecticides may be brought about by decreased cuticular penetration, target site insensitivity, sequestration or metabolic detoxication (Scott 1990).

Detoxifying enzymes were evolved by insects primarily as an evolutionary answer to plant defenses (Krieger et al. 1971, Brattsten et al. 1977). While all insects probably possess some detoxifying abilities, the amount varies among different species, developmental stage and the insect's recent environment. This variation is responsible at least in part for differences in susceptibility to insecticides and the development of insecticide resistance (Terriere 1984). Ahmad et al. (1986) grouped detoxifying enzymes into four categories: oxidases (e.g. mixed function oxidases), hydrolases (e.g. esterases), transferases (e.g. glutathione S-transferases) and reductases (e.g. carbonyl reductases). The mixed function oxidases have
extremely broad spectrum substrates and catalyze a wide variety of biotransformations leading to the metabolism of many insecticides (Nakatsugawa and Morelli 1976). Phosphotriester hydrolases are involved in the metabolism of organophosphorus insecticides (Dauterman 1985). Glutathione S-transferases also metabolize organophosphate insecticides by catalyzing the addition of a tripeptide to the xenobiotic (Motoyama and Dauterman 1980), thus making them water soluble and easily excreted. Carboxylesterases are involved in metabolizing ester-containing insecticides (Matsumura and Brown 1963).

In this chapter, the susceptibility of the yellowmargined leaf beetle to three insecticides as well as the activities of two detoxifying enzymes were investigated. Adult and larval yellowmargined leaf beetle have been reported to be serious defoliators of some cruciferous vegetable ‘green’ crops in southeastern United States (Chamberlin and Tippins 1948). Since the marketability of greens is governed by an absolute lack of feeding damage on the foliage, the principles of economic injury level and economic threshold as espoused by Stern (1973) cannot be applied to insect pest control on these
crops. The judicious use of insecticides will continue to be the most reliable means of achieving quick and effective insect control. At present, there is no insecticide labeled for the control of the yellowmargined leaf beetle on crucifers in Louisiana. Thus, the objectives of the studies reported in this chapter were: (a) study the susceptibility of larval beetle to esfenvalerate, carbaryl and malathion and hence establish a baseline reference point, (b) determine the effect of host plant on beetles susceptibility to insecticides and detoxifying enzyme activities and (c) evaluate the relationship(s) between insecticide susceptibility and detoxifying enzyme activities.

Materials and Methods

**Insecticide susceptibility bioassay.** A topical bioassay was conducted to evaluate the susceptibility of a laboratory population of larval yellowmargined leaf beetle reared on turnip and collard to esfenvalerate (a pyrethroid), carbaryl (a carbamate) and malathion (an organophosphate). These insecticides, though not currently labeled for the control of the beetle in Louisiana, are however labeled for the control of some of the other insect
pests on vegetable green crops. Beetle specimens used in this study were from a laboratory colony that was started in the fall of 1992 from field-collected beetles at the St. Gabriel Research Station, Louisiana State University Agricultural Experiment Station, Iberville Parish, LA.

At the beginning of the study, and at all replications of the assays, about 200 eggs were collected from a beetle colony that has been reared for many generations on turnip foliage. At eclosion, the first instars were divided into two groups and each group was randomly assigned to either turnip or collard. The larvae were fed on the foliage of the assigned plant until they molted into the fourth instar. Bioassays were conducted using 1-day old fourth instars. For each insecticide, a preliminary dose range finding was carried out to determine the lowest and highest concentrations that caused larval mortality. From this range, five graded concentrations of each insecticide were prepared by serial dilutions of technical-grade insecticide samples, using acetone as solvent. The purity and source of the insecticides were carbaryl (98%, Chem Service), malathion (98.5%, Chem Service) and esfenvalerate (DuPont).
For each insecticide and at each replication, sixty larvae were randomly selected and weighed in groups of ten. The groups of ten larvae were randomly assigned to receive one of each insecticide concentration. The sixth group, the control, was treated with acetone. Larvae were placed individually on top of a moistened filter paper in petri dishes and the dishes were labeled with the name of the plant on which the larva was reared, the insecticide treatment and concentration. The assay was done by applying a 1ul aliquot of the insecticide onto the thoracic dorsum of each larva using a 50ul Hamilton® syringe fitted with a blunt needle. After treatment, larvae were supplied with appropriate foliage. Petri dishes containing the larvae were arranged by plant, insecticide and insecticide concentrations in plastic storage boxes. The boxes were arranged in an incubator maintained at 20°C and 14:10 (L:D) h photoperiod. Larvae were checked at 24 and 48 h after treatment for mortality. The criterion for mortality was the inability of a larva to change its position within fifteen seconds after being prodded. The experiment was replicated three times for each insecticide and plant. Larval susceptibility to the insecticides were evaluated by
calculating the LD$_{50}$s for each insecticide at 24 and 48 h after treatment using PROC PROBIT (SAS Institute 1989). The LD$_{50}$s were considered significantly different if the 95% confidence limits do not overlap.

**Enzyme assays.** The activities of two detoxifying enzymes, glutathione S-transferases (GST) and general esterases (EST) toward non-insecticidal substrates were investigated using laboratory reared larvae that have been maintained on turnip and collard. These larvae were handled the same way as those used for the insecticide susceptibility studies. Whole larval homogenates were prepared each time by grinding five 1-day old fourth instars with an all-glass homogenizer in 0.5 ml of 1.15% ice cold KC1 (containing a few crystals of phenythiourea). Homogenates were centrifuged at 10,000g for 10 minutes (at 4°C) and the resulting clear liquid were force filtered through glass wool packed into a funnel.

The activities of GST toward 1-chloro-2,4-dinitrobenzene (CDNB) were measured using the techniques of Jakoby (1978) as modified by Grant et al. (1989, 1991). The enzyme had no activities toward 1,2-dichloro-4-nitrobenzene (DCNB). Buffered CDNB solutions (0.75mM) were
prepared by mixing 305ul stock solutions of CDNB (50mM in
dimethyl sulfoxide) in 20ml phosphate buffer (0.1M, pH 8.0
containing 15% glycerol). Reaction mixtures consisted of
200ul substrate buffer (0.5mM final concentration) and 30ul
enzyme homogenate. A 40ul buffer (0.1M phosphate, pH 8.0)
was added to make a pre-reaction volume of 270ul and
reactions were initiated by adding 30ul reduced glutathione
(GSH)(8mM final concentration). Reaction mixtures were
incubated in flat-bottom microtiter plates (Costar,
Cambridge, MA) at 27°C and the rate of change in optical
density (OD) at 340nm during the initial 10 minutes of the
reaction was measured. A blank plate cell without
homogenate was used as control.

Measurements of the activities of general esterases
(EST) toward alpha naphthyl acetate were made using the
Stock of substrate solution was prepared by dissolving 18mg
of Fast Blue B salt in 30ml of 0.1M phosphate buffer (pH
7.0). Added to this solution was 600ul of 0.113M alpha-
naphtyl acetate dissolved in 50% acetone. The resulting
solution was filtered using a filter paper (Whatman No. 3).
Reaction mixture consisted of 200ul substrate solution and
40ul phosphate buffer (pH 7.0) and 10ul of homogenate.
Reaction without homogenate (200\textmu l substrate solution, 50 \textmu l buffer) served as the control. Reaction mixtures were incubated in flat-bottom microtiter plates (Costar, Cambridge, MA) at 27°C and the rate of change in optical density at 450nm during the initial 10 minutes of the reaction was measured and corrected for non-enzymatic metabolism using incubation without homogenate as control. Enzyme activities were expressed in mOD/min/insect equivalent and mOD/min/mg protein. At each replication of an assay, the amount of protein in the homogenate was quantified using the methods of Bradford (1976). Each enzyme assay was replicated three times by host plant on which the larvae were reared.

Data for enzyme activities were analyzed as a one-way analysis of variance for the effect of plant. Mean enzyme activities by plant were compared using the student’s t-test.

Results

The LD_{50}s for the susceptibility of yellowmargined leaf beetle larvae maintained on turnip and collard to the three insecticides at 24 and 48 h after treatment are reported in Table 7.1. In general, the larvae were most
susceptible to esfenvalerate. For larvae maintained on collard, susceptibility to this insecticide was significantly higher than susceptibility to each of carbaryl and malathion. For larvae maintained on turnip, susceptibility to esfenvalerate was significantly higher than susceptibility to carbaryl and this was also significantly higher than susceptibility to malathion. There were no significant effect of host plant on the susceptibility of the beetle to the insecticides.

The activities of the two detoxifying enzymes, glutathione S-transferases and esterases are reported in Table 7.2. Significant differences were found in the effect of host plant on the activities of GST enzymes of larval beetle maintained on collard and turnip as measured in mOD/min/insect equivalent (F = 6.87, df = 1, P = 0.0587). But there were no significant differences in GST activities measured in mOD/min/mg protein (F = 6.64, df = 1, P = 0.0615). Glutathione S-transferases activities were about 10 times higher in larvae fed collard as compared to those fed turnip. There were no significant differences in the activities of esterases enzymes for beetles fed collard and those fed turnip as measured in
mOD/min/insect equivalent (F = 1.03, df = 1, P = 0.3671)
and in mOD/min/mg protein (F = 1.73, df = 1, P = 0.2591)
(Table 7.2).

Discussion

The yellowmargined leaf beetle is a serious defoliator of cruciferous crops in Louisiana (Oliver and Chapin 1983). At present, there is no insecticide labeled for the control of the beetle despite its state-wide distribution and its damaging impact on some vegetable 'green' crops. This is probably due to the fact that the beetle is an introduced pest that is restricted in distribution to the Southeastern United States. Though there have been reports in the literature on the use of insecticides to control this beetle, none of these reports gave any information about the relative susceptibility of the beetle to the insecticides used. These reports include those of Chamberlin and Tippins (1948), who reported that a heavy dosage of a dust mixture containing 0.75% of rotenone was effective in controlling a small population of the beetle in Alabama. In addition, Woodruff (1974) reported that thiodan (endosulfan) and malathion were labeled for control of the beetle on watercress in Florida. In this study, a
Table 7.1. Susceptibility ($LD_{50}$) of yellowmargined leaf beetle larvae maintained on collard and turnip to carbaryl, esfenvalerate and malathion.

<table>
<thead>
<tr>
<th>Insect Colony</th>
<th>Number Tested</th>
<th>LD50$^a$ (95% CL)</th>
<th>Slope ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carbaryl at 24 h</td>
<td></td>
</tr>
<tr>
<td>Collard</td>
<td>150</td>
<td>0.00812 (0.00517 - 0.01240)</td>
<td>1.25 ± 0.31</td>
</tr>
<tr>
<td>Turnip</td>
<td>150</td>
<td>0.00869 (0.00548 - 0.01394)</td>
<td>1.19 ± 0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carbaryl at 48 h</td>
<td></td>
</tr>
<tr>
<td>Collard</td>
<td>150</td>
<td>0.00589 (0.00285 - 0.00925)</td>
<td>1.07 ± 0.29</td>
</tr>
<tr>
<td>Turnip</td>
<td>150</td>
<td>0.00623 (0.00417 - 0.00846)</td>
<td>1.61 ± 0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Esfenvalerate at 24 h</td>
<td></td>
</tr>
<tr>
<td>Collard</td>
<td>150</td>
<td>7.6 X 10$^{-5}$ (6.3 X 10$^{-5}$ - 9.4 X 10$^{-5}$)</td>
<td>2.76 ± 0.43</td>
</tr>
<tr>
<td>Turnip</td>
<td>150</td>
<td>6.6 X 10$^{-5}$ (5.5 X 10$^{-5}$ - 7.7 X 10$^{-5}$)</td>
<td>3.37 ± 0.48</td>
</tr>
</tbody>
</table>

Table 7.1. (continued)
<table>
<thead>
<tr>
<th>Insect Colony</th>
<th>Number Tested</th>
<th>LD50&lt;sup&gt;a&lt;/sup&gt; (95% CL)</th>
<th>Slope ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Esfenvalerate at 48 h</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collard</td>
<td>150</td>
<td>$6.1 \times 10^{-5}$ ($4.9 \times 10^{-5} - 7.6 \times 10^{-5}$)</td>
<td>$2.56 \pm 0.41$</td>
</tr>
<tr>
<td>Turnip</td>
<td>150</td>
<td>$5.7 \times 10^{-5}$ ($4.7 \times 10^{-5} - 6.9 \times 10^{-5}$)</td>
<td>$2.98 \pm 0.43$</td>
</tr>
<tr>
<td><strong>Malathion at 24 h</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collard</td>
<td>150</td>
<td>$0.08979$ ($0.06567 - 0.16026$)</td>
<td>$1.69 \pm 0.39$</td>
</tr>
<tr>
<td>Turnip</td>
<td>150</td>
<td>$0.11117$ ($0.08457 - 0.18268$)</td>
<td>$2.30 \pm 0.47$</td>
</tr>
<tr>
<td><strong>Malathion at 48 h</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collard</td>
<td>150</td>
<td>$0.05497$ ($0.04093 - 0.07861$)</td>
<td>$1.69 \pm 0.36$</td>
</tr>
<tr>
<td>Turnip</td>
<td>150</td>
<td>$0.09292$ ($0.06898 - 0.15819$)</td>
<td>$1.84 \pm 0.39$</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as ug insecticide/larva
Table 7.2. The activities of glutathione S-transferases and esterases enzymes of yellowmargined leaf beetle larvae fed collard and turnip.

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Glutathione S-transferases</th>
<th>Esterases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insect equivalent</td>
<td>mg protein</td>
</tr>
<tr>
<td>Collard</td>
<td>174.67 ± 64.01</td>
<td>80.74 ± 28.07</td>
</tr>
<tr>
<td>Turnip</td>
<td>6.79 ± 2.02</td>
<td>8.07 ± 2.73</td>
</tr>
</tbody>
</table>

Enzyme activities in mOD/min (Mean ± SE)
laboratory toxicological study was conducted for the first time to determine the susceptibility of the beetle to commonly used insecticides and establish a reference point to detect and monitor insecticide resistance in field populations. In general, susceptibility of the larvae to the insecticides was esfenvalerate > carbaryl > malathion. Variations in the susceptibilities of different insects to the different classes of insecticides have been reported in the literature. For instance, the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was reported to be more susceptible to permethrin and least susceptible to carbaryl and malathion (Hare 1980). On the other hand, Weiss et al. (1991) reported that the flea beetle, *Phylotreta cruciferae* (Goeze), was more susceptible to carbaryl and least susceptible to malathion, with susceptibility to esfenvalerate lying between the two extremes.

Factors accounting for the differential response of the yellownarrowined leaf beetle to the different insecticides were not accounted for in this study. It is clear however that the plant on which the beetle was reared did not account for the differences in insecticide susceptibility. There were no significant differences in
the susceptibilities of beetles reared on collard as compared to those reared on turnip. Some authors have reported that the diet of some phytophagous insects sometimes significantly affects their susceptibilities to insecticides. Ghidiu et al. (1990), for instance, reported that Colorado potato beetles reared on eggplant were significantly more susceptible to permethrin than those reared on tomato. They suggested that variations in the allelochemical contents of the plants might be responsible for these differences. In general, the same enzyme systems are used to detoxify plant allelochemicals and insecticides (Moldenke et al. 1992). Thus it is expected that insecticide susceptibility is affected by the level and activities of detoxifying enzymes brought about by variations in the allelochemical contents of the plants.

There was a marginal significance in the effect of host plant on the activities of the glutathione S-transferases enzymes. The activities of this enzyme was about 10 times higher for larvae fed collard than those fed turnip. Considering the fact that larvae used in these assays were started originally on turnip before some were switched onto collard, and that those switched onto
collard had higher enzyme activities implies that collard
probably contains chemical compounds that were responsible
for causing the elevated activities. Elevated activities
of a detoxifying enzyme called enzyme induction has been
reported in the cutworm, Peridroma saucia Hübner, (Yu et
al. 1979) and the fall armyworm, Spodoptera frugiperda
(J. E. Smith), (Yu 1982). In the fall armyworm, it was
reported that host plants such as cowpeas, turnip and
mustard induced the glutathione S-transferase enzymes 7 to
10-fold as compared to cotton, corn, soybean and
bermudagrass (Yu 1982).

The elevated level of detoxifying enzymes in beetles
fed collard did not however have any significant effect on
their susceptibility to insecticides. This result is
similar to that obtained by Kirby et al. (1994) who studied
the activities of the two enzymes in the tobacco budworm,
Heliothis virescens (F.). They reported that there were no
correlations between enzyme activities and susceptibility
to a pyrethroid insecticide in insecticide resistant
budworm larvae. This lack of correlation was seen as
suggesting some limitations in the use of noninsecticidal
substrates as indicators of metabolic resistance to
insecticides.
Elevated activities of detoxifying enzymes in yellowmargined leaf beetle larvae switched from turnip onto collard might be important in explaining differences in behavior and physiological fitness of beetles reared on these hosts. Beetles fed collard were significantly less fecund (198.85 ± 28.94) than beetles fed turnip (490.74 ± 116.04) (See Chapter four). In addition, the beetle showed a strong feeding preference for the foliage of turnip over that of collard (See Chapter five). From the foregoing, it is tempting to speculate that collard probably contains toxic allelochemical(s) which are absent in turnip. These chemicals, whose identity is not yet known makes collard less attractive for feeding and beetles forced to feed on collard reacted by using a larger proportion of their metabolic resource toward producing detoxifying enzymes to deal with these hostile chemistries. The production of detoxifying enzymes always results in some metabolic costs to the insect (Schoonhoven and Meerman 1978, Brattsten 1979, Appel and Martin 1992). The idea of a metabolic cost to the insect brought about by the plant on which the insect was raised has been reported for the Colorado potato beetle (Ghidiu et al. 1990). Colorado potato beetles
reared on tomato were reported to be smaller and less fecund than those reared on potato. This was attributed to variations in the α-tomatine content, a steroidal glycoalkaloid found in the tissues of many members of the genera *Lycopersicon* and *Solanum*. There is thus the need to study the allelochemical profiles of cruciferous plants in relation to the fitness of the yellowmargined leaf beetle.
REFERENCES CITED


Hare, J. D. 1980. Contact toxicities of ten insecticides to Connecticut populations of the Colorado potato beetle. J. Econ. Entomol. 73: 230-231.


Painter, R. H. 1951. Insect Resistance in Crop Plants. University of Kansas, Lawrence, KS.


VITA

Abdullahi Oloduowo Ameen was born in Lagos, Nigeria sometimes after the British departed his country. He was raised by his grandparents, Imam Mustafa and Hajia Bilikisu, who made sure he received an early Koranic education in his hometown, Ilorin, Nigeria. He got his B.S. and M.S. degrees in Zoology from the University of Ilorin, Ilorin, Nigeria. Between 1987 and 1992, he was a lecturer at the Department of Biological Sciences, Bayero University, Kano, Nigeria. He came to the Louisiana State University for graduate studies in June 1992. Currently he is a candidate for the Doctor of Philosophy degree in the Department of Entomology.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Abdullahi O. Ameen

Major Field: Entomology

Title of Dissertation: The Biology and Ecology of the Yellowmargined Leaf Beetle, Microtheca ochroloma Stal, (Coleoptera: Chrysomelidae) on Crucifers

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: March 29, 1996