
Lilia Ibay De guzman

Louisiana State University and Agricultural & Mechanical College

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Tolerance potential and defense mechanisms of honey bees (*Apis mellifera* L.) to *Varroa jacobsoni* Oud. (Acari: Varroidae) and *Acarapis* species (Acari: Tarsonemidae)

de Guzman, Lilia Ibay, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1994
TOLERANCE POTENTIAL AND DEFENSE MECHANISMS
OF HONEY BEES (APIS MELLIFERA L.) TO
VARROA JACOBSONI OUD. (ACARI: VARROIDAE) AND
ACARAPIS SPECIES (ACARI: TARSONEMIDAE)

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

Lilia Ibay de Guzman
B. S., Central Luzon State University, 1981
M. S., Oregon State University, 1989
August 1994
DEDICATION

To my parents, Mr. Juan P. Ibay and Mrs. Aquilina
Armendez Ibay, this dissertation is dedicated.
ACKNOWLEDGMENTS

I want to thank my major professor, Dr. Thomas E. Rinderer, for his guidance throughout this endeavor. My productive years as a student were due to his encouragements, trust and endless support.

To my committee members: Dr. Jerry B. Graves, Dr. Frank Guillot, Dr. John Harbo, Dr. Dorothy Pashley and Dr. Marion Socolofsky, thank you for your assistance and suggestions to the manuscripts.

I wish to express my appreciation for the statistical assistance of Ms. Vicki Lancaster, Dr. Raul Macchiavelli, Dr. Steve Buco and Dr. Deborah Boykin.

I also want to acknowledge the generosity of Horace and Louella Bell, owners of the Bell's Honey Company in De Land, Florida for providing all the colonies as well as bee feed and equipment used in the Florida portions of this study. And thanks also to Mr. Griggs, Mr. Godwin, Mr. Owens and Mr. Gibbs for giving me the permission to use their land as experimental sites.

I am very much indebted to the technical staff of the Honey-Bee Breeding, Genetics and Physiology Research Laboratory: Lorraine Beaman, Gary Delatte, Daniel Pursifull, James Pursifull, Anthony Stelzer and Dan Winfrey for their help with the data gathering. And to all the employees of the Bee laboratory, thank you for the professional help, for being very patient in attending to
my needs and most of all for the friendship. Special thanks to Lorraine Beaman for making all the graphics.

I am grateful to all my friends in Oregon, the Guidry family and Filipino students at LSU for their continued encouragements, concern and everlasting friendship. The help provided by Asun, Racquel, Abel and Freddie is deeply appreciated.

To the Ibay and de Guzman families, thank you for the prayers, understanding and moral support. Special thanks to my sister Ely who worked hard to finance my college education. Without her support I could not have reached this far.

Most of all to my family, especially to my husband, Cris whose love and understanding made this program possible. And to my three children Sheila, Lorena and Marlou, thanks for your smiles, hugs and cooperation. You were my inspiration.
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Comparative resistance or tolerance of four stocks of *Apis mellifera* L. to *Varroa jacobsoni* Oudemans, *Acarapis dorsalis* Morgenthaler, *Acarapis externus* Morgenthaler and *Acarapis woodi* (Rennie), was investigated using choice (bioassay) and field experiments. *A. m. carnica* from Yugoslavia (ARS-Y-C-1), *A. m. carnica* from Canada (Hastings), F₁ hybrid between ARS-Y-C-1 and Hastings and a general Louisiana stock were evaluated.

A tolerance index showed that Hastings, ARS-Y-C-1 and F₁ hybrid exhibited some degree of tolerance to *V. jacobsoni*. These stocks lived longer with higher levels of *Varroa* infestation in worker pupae and on adult honey bees. Louisiana stock was more susceptible to *Varroa* infestation showing an earlier death with lower levels of infestation.

Regardless of the stock, *Varroa* had similar reproductive success based on the number of mites per infested pupa (1 to 10 mites), number of foundress *Varroa* per infested pupa (1 to 3 females), number of progeny per female (0 to 5 progeny), number of progeny per infested pupa (0 to 7 progeny), and proportion of infested pupae containing infertile foundresses (0 to 47%). Apparently, differences in the duration of the capped stage of the four stocks did not influence the ability of *Varroa* to reproduce. ARS-Y-C-1 stock had the longest capped period
and the shortest was observed in the F₁ hybrid. Louisiana stock seemed to have a better grooming behavior than the three other stocks as shown by the higher number of recovered dead Varroa mites. However, this characteristic did not influence the stock's ability to tolerate mite infestations.

ARS-Y-C-1 and the F₁ hybrid also showed considerable resistance to A. woodi. Colonies of these stocks consistently maintained about 10% tracheal mite infestations in two field trials. This level is well below the level (25%) reported to cause economic damage in honey bees. Using a bioassay, resistance to A. woodi displayed by ARS-Y-C-1 stock was comparable to that of Buckfast and their reciprocal hybrids. Louisiana and Hastings stocks had the highest levels of tracheal mite infestation. A. dorsalis was most prevalent in the Hastings stock and levels of A. externus were higher on ARS-Y-C-1, F₁ hybrid and Louisiana stocks.
INTRODUCTION

In the United States, four species of mites are known to be parasitic to honey bees (Apis mellifera L.): Varroa jacobsoni Oudemans, Acarapis dorsalis Morgenthaler, Acarapis externus Morgenthaler and Acarapis woodi (Rennie). Of these, V. jacobsoni and A. woodi are considered serious threats to the American beekeeping industry.

Varroosis on A. mellifera colonies causes wing deformation and weight and size reduction of infested bees (De Jong et al. 1982, Engels & Schatton 1986). Occasionally, it results in the death of the developing brood of A. mellifera (Akratanakul & Burgett 1975). This parasite also acts as a vector of Acute Paralysis Virus (APV) of honey bees (Ball 1985, 1988; Carpana et al. 1990).

Since its discovery in Texas in 1984 (Delfinado-Baker 1984), acarapisosis has been linked with tremendous colony losses in the U. S. High levels of A. woodi infestation reduced honey yield and caused poor wintering ability (Eischen 1987, Eischen et al. 1989). Lifespan of honey bees is also reported to be shortened (Bailey 1958, Maki et al. 1988, Royce & Rossignol 1990).

Mite parasitism on honey bees is a great threat to agriculture. This is especially so in countries like the U. S. where honey bee pollination services are required
for crop production. In the U. S., 7 to 10 billion dollars of agricultural production is totally or partially dependent upon honey bee pollination. This amounts to approximately one third of the country's human food consumption (McGregor 1976). Effective control measures for parasitic mites of honey bees clearly are necessary to sustain effective pollination by honey bees.

The usual approach to mite control is chemical treatment. Many chemicals have been tested against these bee parasites but nothing has shown complete control. A difficulty in controlling *Varroa* mites is due in part to the restriction of reproductive mites inside the brood cells. In the U. S., Apistan is the only registered chemical for beekeepers to use. For the control of tracheal mites, menthol is effective and legal. The increased use (sometimes illegally) of acaricides has raised concerns of health hazards and hive product contamination. Because mites have multiple generations per year, frequent use of chemicals will increase selective pressure on the mite populations and acaricide resistance is likely to develop.

Natural resistance to *V. jacobsoni* by honey bees has been reported. Peng et al. (1987) observed cleaning behavior of *Apis cerana* F. against this parasitic mite; adult bees remove mites from other adults and kill them. In *A. mellifera* carnica Pollman, workers bees were
observed to remove mites together with the infested brood (Boecking & Drescher 1991).

The genetic make-up of the bee host has been suggested to influence its resistance to this ectoparasite (Moritz & Hanel 1984, Moritz 1985, Camazine 1986, 1988). Accordingly, European bees are thought to be more susceptible to mite infestation because of a longer postcapping duration than African bees (A. m. capensis). However, De Jong et al. (1984) contradicted this view and suggested that the resistance of Africanized honey bees (descendants of A. m. scutellata) is due to climatic reasons since European bees also seem to resist varroosis in Brazil.

Breeding honey bees which are resistant or tolerant to parasitic mites then is a promising long term solution to these global threats. Improved methods of evaluating honey bees for mite resistance should therefore be developed and mechanisms of resistance be identified for future use in breeding programs.

Four generations of divergent selection in a Yugoslavian A. m. carnica population resulted in two lines that varied in their levels of mite infection (Kulinčević & Rinderer 1988, Kulinčević et al. 1992). Selection was based on the average numbers of pupae infested and also the average number of dead mites resulting from fluvalinate fumigation. Evidence of resistance to Varroa
was observed. However, whether or not the level of resistance was economic was not established. In August 1989, daughter queens of the fourth generation of selection were imported into the U. S. for further study (de Guzman et al. 1990, Rinderer et al. 1993).

This dissertation evaluates the potential for tolerance or resistance of the imported Yugoslavian stock, called ARS-Y-C-1, to *V. jacobsoni* and three *Acarapis* species (*A. dorsalis*, *A. externus* and *A. woodi*) in comparison to three selected stocks of honey bees. In addition, the potential mechanisms of resistance to Varroa mites by the stocks are discussed. Chapter I examines the effectiveness of a bioassay or short test for the evaluation of resistance to Varroa mites by the different stocks of honey bees. Chapter II compares the tolerance of four stocks of honey bees to *V. jacobsoni* under field conditions. Chapter III analyzes the differences in the capped duration of the bee stocks as a potential mechanism of resistance to Varroa mites. This chapter also explores the ability of Varroa deutonymphs to survive and develop through adulthood when inoculated into newly capped larvae. Chapter IV measures the resistance of the different bee stocks to the three *Acarapis* species with emphasis on tracheal mite resistance. Chapter V compares the *A. woodi* resistance of ARS-Y-C-1 stock with that of the Buckfast stock and their reciprocal hybrids.
References


Mites (Acari) are the major limiting factor to a successful beekeeping industry. There are several mite species known to be parasitic to honey bees. These mites belong to the families Varroidae, Laelapidae (order Parasitiformes) and Tarsonemidae (order Acariformes). *V. jacobsoni* belongs to the family Varroidae while *Acarapis* species (*A. dorsalis, A. externus* and *A. woodi*) belong to the family Tarsonemidae.

The *Varroa* mite is originally a parasite of the Asian hive bee, *A. cerana*, but successfully switched to the western hive bee, *A. mellifera*, when it was brought to Asia in the 1950's. Recently, *V. jacobsoni* was found in colonies of *A. koschevnikovi* (Buttel-Reepen) in Borneo (Delfinado-Baker et al. 1989). The *Acarapis* mites on the other hand, are specific to *A. mellifera*.

The geographical ranges of *V. jacobsoni* are evidently expanding. The colonization ability of this parasitic mite is influenced by several factors. Such factors include hormonal titres of bees, climate, cell size and also management practices employed on the bee colonies (Ritter 1988). Other factors affecting the growth of mite populations are: (1) the reproductive ability of the female, (2) the ability of mites to infest new colonies, (3) postcapping duration, and (4) race-specific characteristics of honey bees (Otten 1990).
Resistance to *V. jacobsoni* by honey bees has been reported by several researchers. After four generations of selection, resistance to this parasite was observed in the Yugoslavian honey bees, *A. m. carnica* (Kulinčević & Rinderer 1988, Kulinčević et al. 1992). However, whether or not the level of resistance was economic was not established.

For tracheal mite resistance, Buckfast stock was developed by Brother Adams in England and is commercially available in the U. S. The mechanisms of resistance however, have not been identified. The resistance of Buckfast stock to *Varroa* has yet to be established.

Resistance mechanisms have been demonstrated in some *Apis* species against their adapted parasites. On its original host, *A. cerana*, *V. jacobsoni* infestation and reproduction is very low. Peng et al. (1987a) correlated this resistance to the cleaning behavior of the bee. The irritation of infested hosts alerted bees to groom and eventually removed phoretic mites from the worker bees. When infested brood of *A. mellifera* was reared inside *A. cerana* colonies, the latter removed the mites from the adult bees of the former (Peng et al. 1987b). Cleaning behavior was likewise observed in European bees but not frequently seen.

The "lycurgan" behavior, that is, the removal of the infested developmental instars of hosts together with
the mites, has been postulated by Burgett et al. (1990) on *A. dorsata* F. The authors believed that workers could monitor *T. clareae* Delfinado and Baker and subsequently remove infested brood along with the mites. Observations of similar behavior with *V. jacobsoni* were reported by Boecking and Drescher (1991) for *A. mellifera*. Test bees were observed to remove 14.3 to 95.8% of the brood infested with one *V. jacobsoni* and 25 to 100% removal of brood infested with two mites.

The reproductive success of *V. jacobsoni* is also affected by brood gender of bee hosts. Like *E. sinhai* Delfinado and Baker on *A. florea* F., *V. jacobsoni* exclusively reproduce in drone brood of *A. cerana* (Akratanakul & Burgett 1975, Koeniger et al. 1981). However, in Korea, reproduction in worker brood was noted by De Jong (1988). The same observation was reported by Sasaki (1989) on *A. cerana japonica* in Japan.

Although *V. jacobsoni* infests both worker and drone brood in *A. mellifera*, drone brood is preferred 3-8 times more than worker brood (Ritter & Schneider-Ritter 1988, Woyke 1990). The same observation was reported by Ruttner and Marx (1984) in Uruguay. Reproductive factors of fertile females were reported by Schulz (1984) as 1.8 and 2.7 for worker and drone brood, respectively.

*A. woodi* also infests all castes including queens (Pettis et al. 1989). However, the level of infestation
in drones is higher than in queen or worker bees. Thus, drones act as an important sanctuary of tracheal mites in the colony (Royce & Rossignol 1991). In worker bees, an adult female A. woodi can lay about 5-10 eggs (Morgenthaler 1931, Bailey 1963, Delfinado-Baker 1988, Royce et al. 1988). A much higher estimate of 21-25 progeny per female was postulated by Pettis (1991). The external Acarapis species have lower reproductive capabilities. Females lay an average of two to three eggs (Royce et al. 1988). Infestations of the external Acarapis on drones has not been explored.

The population growth of Varroa is dependent on the proportion of infertile females infesting the cells (Otten 1990). The author claimed that the proportion of infertile females fluctuates through time. He observed that during January to February in Germany, 65% infertile females was recorded in colonies of A. m. carnica while 75% was noted in A. m. ligustica Spinola colonies. The proportion of infertile females declined from March to November with the lowest proportion (11%) recorded during May to August in the carnica type. An A. ligustica strain had the lowest proportion of 15% from May to November. Ritter et al. (1990) observed about 30-50% infertile females in worker brood of Tunisian honey bees. The same authors also observed a similar proportion of infertile females in the drone brood. A lower proportion of 5-13%
infertile females was recorded in A. m. carnica colonies in Yugoslavia (Sulimanovic et al. 1986, Kulinčević et al. 1988). A comparable result (14-18%) was obtained by Ifantidis (1990) in Greece using A. m. macedonica colonies. These infertile mites are probably adult females that are not fertilized prior to bee emergence.

The reproductive ability of V. jacobsoni in different races of bees was also studied by other researchers. However, contradictory results have been obtained. A lower rate of reproduction was observed in Africanized bees than in A. m. carnica (Engels et al. 1986, Moritz & Mautz 1990). The same observation was reported by Camazine (1986). Accordingly, only 49% of females produced progeny in the Africanized bees while 75% reproduced in the European colonies. In the European worker brood, a female Varroa produces 4-7 progeny per infested cell with 1-2 females reaching adulthood (Schulz 1984, de Ruijter 1987, Woyke 1990). Kulinčević and Rinderer (1988) observed reproductive rates ranging from 1.41 to 3.8 in A. m. carnica colonies in Yugoslavia. However, the reproductive success of A. m. carnica in the tropics is lower than in Europe (Engels & Schatton 1986). The authors postulated that there may be a differential climatic tolerance among species or races of honey bees.

The importance of climate in the reproductive success of V. jacobsoni was suggested by De Jong et al.
They argued that this success was independent of bee race. Additionally, a lower infestation was observed in the hotter regions of South America (Ruttner & Marx 1984). However, in the cooler region of Brazil, where most colonies are Africanized, higher infestations were noted (De Jong et al. 1984).

The population of *Acarapis* mites in a colony also depends on the genotypes of the honey bee. The prevalence of *A. woodi* in two different stocks of *A. mellifera* was compared by Milne et al. (1991). The authors claimed that Buckfast (Texas) stock was more resistant to tracheal mites having a lower mite prevalence and a mite load than the California stock. Buckfast stock is a result of the selection program done by Adams (1987). No similar studies have been done with the external *Acarapis*.

The phenology of the two external *Acarapis* varies from location to location. In Massachusetts, Shaw et al. (1961) observed the highest external *Acarapis* infestations in spring and summer. The same pattern was observed in Western Canada (Clark 1985). Eckert (1961) found that *A. dorsalis* had peaks of infestation during spring and fall in California. For *A. externus*, a wide fluctuation in the infestation was observed. In Oregon, *A. dorsalis* infestation was highest in the spring months (March to June) when suitable hosts are emerging. The lowest
Infestations were observed in January when no young bees are available and in July coinciding to the peak of adult bee population thereby diluting the mite population in the hive. The highest *A. externus* infestation was noted in the fall (October and November) when relatively old bees populate the hive. A separate experiment showed that this external *Acarapis* can maintain its population in older hosts. Infestation was lowest during the month of July (Ibay 1989, de Guzman & Burgett 1990). In New Zealand, populations of the external *Acarapis* also decrease in winter and summer (Clinch 1975).

One of the mechanisms affecting virulence of *V. jacobsoni* is the length of developmental period of various bee species and races (Moritz & Hanel 1984, Moritz 1985). They observed that African cape bees (*A. m. capensis*) have a shorter (9.7 days) capped duration than European honey bees (11.5 days). A similar value was reported by Harbo (1992) for the postcapping period of European honey bees. Moritz and Hanel (1984) also showed that this characteristic is heritable. Therefore, determining the duration of the postcapping stage of various *A. mellifera* stocks will be very useful in the development of a honey bee strain resistant to *V. jacobsoni*.

Since breeding of honey bees resistant to parasitic mites offers long term, inexpensive and non-hazardous control, behavioral and physiological
differences in resistance or tolerance are very important to consider as potential characteristics for selection programs.
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CHAPTER I

A SHORT TEST EVALUATING RESISTANCE OF HONEY BEES (APIS MELLIFERA L.) TO VARROA JACOBSONI OUDEMANS (ACARI: VARROIDAE)
Introduction

*Varroa jacobsoni* Oudemans is a serious ectoparasite of *Apis mellifera* L. Heavy infestations of this parasite will result in morphological and physiological deformities of emerging adult honey bees which ultimately lead to the collapse of infested colonies (de Jong et al. 1982, Engels & Schatton 1986). Since its discovery in the United States in 1987, infestations of *Varroa* are causing tremendous economic damage to apiculture across the country. In order to reduce mite damage, beekeepers rely on routine applications of chemicals. In the U. S., Apistan is the only chemical available and legal to use. Certainly, any chemical use raises concerns of hive product contamination. Also, ubiquitous and relentless use of this chemical for several years may produce mite populations that are resistant to this miticide. The increasing restrictions on the availability of acaricides and the possible development of resistant mite populations have created an immediate need for an alternative control strategy.

The increasing efforts to find honey bees resistant to *Varroa* mites have been very encouraging. In Germany, resistance to *Varroa* has been observed in different subspecies of *A. m. mellifera* (Boecking & Drescher 1991, Moritz & Mautz 1990, Otten 1990, Ruttner & Hanel 1992). In Yugoslavia, *A. m. carnica* Pollman has
been shown to have some degree of resistance to Varroa (Kulinčević & Rinderer 1988, Kulinčević et al. 1992).

The Yugoslavian A. m. carnica stock, called ARS-Y-C-1, was imported to the U.S. through quarantine for further testing (de Guzman et al. 1990, Rinderer et al. 1993). The evaluation of ARS-Y-C-1, although including data collection on other characteristics, focused on response to Varroa jacobsoni infestation.

Typically, assessments of response to Varroa involve extended field evaluation. Such evaluations are costly and time consuming. It would be desirable to have a bioassay or short test which could provide important information on some broad measure of resistance or tolerance. This study was conducted to: 1) develop a simple method for testing resistance to V. jacobsoni, and 2) to compare the resistance of ARS-Y-C-1 to Varroa with selected stocks of A. mellifera using the simple test.

Materials and Methods

Daughter queens of ARS-Y-C-1 (A. m. carnica), which were known to be somewhat resistant to Varroa, were imported into the U.S. from Yugoslavia in August 1989 to determine if the level of resistance exceeded that of representative U.S. commercial stocks of honey bees. Consequently, ARS-Y-C-1 was compared to three selected stocks of A. mellifera: Hastings (A. m. carnica) from Northern Saskatchewan, F1 hybrids between ARS-Y-C-1 and
Hastings stocks and a general Louisiana stock. Queens from each stock were instrumentally inseminated with 8μl of mixed semen. This study was conducted in September 1992 at the Baton Rouge Bee Laboratory.

In order to compare the attractiveness of the four stocks to Varroa infestations, uncapped larvae from each test stock were exposed to Varroa mites simultaneously. This method provided larvae of all stocks with similar chances of infestation and was achieved by grafting or transferring young larvae of all test stocks into a section of a brood frame. A grafting technique was used by Harbo (1992) to compare the developmental time of honey bee stocks. Harbo's method was used in the present study with few modifications, except that infestation variables rather than development times were observed.

Prior to grafting, two colonies with known Varroa infestations (20 and 24%) were established as host colonies or colonies that received the grafted larvae which are referred to here as target larvae. A total of five trials (five colonies in each stock) were done; two trials in the colony with 20% Varroa infestation and three trials in the colony with 24% infestation. Since Varroa infests older larvae, some frames containing old uninfested larvae were removed from the colonies to channel mites available for infestation toward the target larvae.
In each test colony, an empty frame was inserted at the middle of the brood nest for egg laying. Frames were inspected the next day for the presence of eggs which were later used in grafting. This procedure was conducted three days thereafter when the eggs had hatched and the larvae were less than one day old. At this time, a brood frame with young larvae from each host colony was chosen to receive the grafted larvae. Colonies were fed with sugar syrup and pollen patties one week prior to grafting to increase brood rearing.

At the center of a frame receiving the brood, an area occupying 8 rows of 20 cells was established. Two rows were assigned randomly for each stock per trial. Larvae inside the cells within the area were discarded and cells were cleaned using a grafting needle. A drop of royal jelly was then placed at the bottom of the cells and larvae from each stock (approximately one day-old) were then grafted into the cells. Priming of royal jelly was done one row at a time to prevent the material from drying. The grafted brood was then mapped onto a transparent sheet and the frame was placed back in the host colony.

After two weeks, when cells contained dark-eyed pupae (about 16-17 days old) (Ifantidis 1984), all cells occupied with pupae were examined for the presence of mites. Mites together with the pupae were placed in
Eppendorf vials containing 70% ethyl alcohol for later examination under a dissecting microscope. Numbers of mites infesting a pupa were counted and all developmental stages were differentiated.

Resistance predictors examined were: 1) proportion of pupae infested, 2) number of mites per infested pupa (mite load) which included all the different developmental stages, 3) number of foundress Varroa, which were distinguished from daughter females based on the color of the idiosoma; darker-colored females were regarded as the mother mites and daughters were the lighter-colored females, 4) number of progeny per foundress Varroa, 5) number of progeny per infested pupa, and 6) proportion of infested pupae with infertile females or foundresses with no progeny at the time of observation.

Data on the percentages of pupae infested with Varroa were analyzed using a Logit Linear Model. Data were transformed by logit transformation. This transformation adjusted for the non-normal error structure of the binary response of individual bees either being infested or not infested. This non-normal error structure caused the variance to depend upon the mean, a condition which is adjusted by the transformation. The Categorical Data Modeling Procedure was used to conduct an analysis of variance and subsequent contrasts of mean responses using
Chi-square. All other variables were analyzed using a Randomized Block Design with trials considered as blocks and stocks as treatments using the General Linear Model Procedure (SAS Institute, Inc. 1990).

Results

The proportion of cells infested with *V. jacobsoni* significantly differed ($\chi^2 = 9.08; \text{df} = 3; P < 0.0283$) among the stocks tested (Figure 1.1). Analysis revealed that level of *Varroa* infestation in the ARS-Y-C-1 stock was the lowest. The highest levels of *Varroa* infestation was observed in the Hastings and Louisiana stocks, which were different from that of ARS-Y-C-1 stock. F$_1$ hybrid was intermediate between its parental ARS-Y-C-1 and Hastings stocks. Louisiana, Hastings and F$_1$ hybrid stocks were not statistically different.

The number of mites per infested pupa showed no differences among the stocks ($P < 0.6695$) (Appendix A.1). An infested pupa contained about 4-6 mites, mostly of different stages. No significant differences were detected for the number of foundress *Varroa* per infested pupa ($P < 0.6593$) (Appendix A.2). About 1-2 foundress *Varroa* were recorded per infested pupa. The ability of a female mite to reproduce ($P < 0.1745$) and the number of progeny found in an infested pupa ($P < 0.8106$) also showed no significant differences among the test stocks (Appendix A.3). A female *Varroa* was able to produce 3-4 progeny.
Figure 1.1. ANOVA and graph of the percent infestation of *Varroa jacobsoni* in four selected stocks of *Apis mellifera* evaluated for resistance using a bioassay. Bars with different letters indicate means are significantly different (Contrast, SAS Institute, Inc. 1990).
However, inside an infested pupal cell, about 4–5 progeny were observed (Appendix A.3). It is possible that these progeny may have been produced by more than one female present in the cell. There was no significant influence of stocks on the proportion of infested pupae containing infertile Varroa females (P< 0.5858)(Appendix A.5). Proportions of infertile females ranged from 2.8±9.9 to 22±9.9% which were observed in the Louisiana and Hastings stocks, respectively.

Discussion

Reproductive success of V. jacobsoni in different A. mellifera subspecies has been studied by several researchers under field conditions. In this study, a bioassay was used. Giving the Varroa mites a choice, we observed that ARS-Y-C-1 (A. m. carnica) and the hybrid colonies were less attractive to Varroa infestations as shown by the lower proportions of pupae infested. The percentage (23.65%) of pupae infested for ARS-Y-C-1 was lower than the final infestation rate (40%) of one of the parents used in the selection for the first generation of this stock (Kulinčević & Rinderer 1988). This observation may suggest that ARS-Y-C-1 colonies acquire lower infestations of Varroa upon exposure to the mite and mite populations slowly increase over time.

It is also interesting to note that the F₁ colonies were intermediate between their ARS-Y-C-1 and
Hastings parents. This suggests that the underlying genetic difference between ARS-Y-C-1 and Hastings stocks may be a simple trait lacking dominance.

The preference or nonpreference of mites to host bees may be attributed to the existence of chemicals which may attract or repel a particular mite species. Trouiller et al. (1992) reported that high levels of kairomones were present in the brood prior to capping in *A. mellifera*. Whether or not the levels of kairomones were different in all the stocks tested, which may have caused the discrepancy, is not known.

However, we observed that once a brood cell was infested, the reproductive ability of *Varroa* was similar for all the stocks. After years of selection, the reproductive rate displayed by *A. m. carnica* in Yugoslavia ranged from 1.41 to 3.8 (Kulinčević & Rinderer 1988). The results of the present bioassay showed similar reproductive ability of *Varroa* in the ARS-Y-C-1 stock as shown in the number of progeny per foundress which was about four progeny. The number of progeny per infested pupa was recorded to be about 4-5 progeny for all the stocks, which may have been produced by more than one foundress.

Under the condition of a choice experiment, ARS-Y-C-1 stock and its F₁ hybrid demonstrated their potential for resistance to *V. jacobsoni*. ARS-Y-C-1 was selected
for reduced infestation rates (Kulinčević et al. 1992), a trait of the stock confirmed by this test. Consequently, our data suggest that the short test is an effective bioassay for evaluating resistance to Varroa mites. Field evaluations of the same stocks are discussed in Chapter II.
References


CHAPTER II

EVALUATION ON THE TOLERANCE OF SELECTED
STOCKS OF APIS MELLIFERA L. TO
VARROA JACOBSONI OUDEMANS (ACARI: VARROIDAE)
Introduction

Increasing mortality of *Apis mellifera* L. colonies due to the feeding activities of *Varroa jacobsoni* Oudemans is a serious problem worldwide. Because of the negative effects of using acaricides, such as potential effects on honey bees, contamination of hive products and the development of acaricide resistance in mites, alternative control of *Varroa* such as the use of resistant stocks of honey bees is desirable. This strategy may be the only hope for a long term solution to this global threat.

Attempts to find stocks of honey bees resistant to *Varroa* have been pioneered by Europeans. In the United States, research towards this objective has been limited. Four generations of divergent selection in a Yugoslavian *A. m. carnica* Pollman population resulted in two lines that varied in their levels of mite infection (Kulinčević & Rinderer 1988, Kulinčević et al. 1992). In August 1989, daughter queens of the fourth generation of selection were imported into the U. S. for further study (de Guzman et al. 1990, Rinderer et al. 1993). The present study was conducted to determine if the level of resistance achieved by the stock named as ARS-Y-C-1 exceeded that of the U. S. honey bee populations and if the level of resistance was sufficient to maintain commercial colonies without chemical treatment.
Materials and Methods

The degree of resistance of ARS-Y-C-1 (A. m. carnica from Yugoslavia) was compared to three other stocks: Hastings (A. m. carnica) from Northern Saskatchewan, F₁ hybrids between ARS-Y-C-1 and Hastings stocks and a general Louisiana stock. About thirty experimental queens were reared from each stock. Each queen was instrumentally inseminated with appropriate semen (8 μl/queen). Drones for the insemination of Louisiana queens were collected using an aerial drone trap (Taylor 1984) set at the Baton Rouge laboratory apiary.

The study involved two experimental trials. Trial 1 was initiated in De Land, Florida in June 1990. For better acceptance of queens, colonies were left queenless for a day prior to the introduction of queens from respective stocks. Test colonies were moved to Panama City, Florida in November 1990 and to Pensacola, Florida in June 1991. Two apiary sites were used with 10 colonies per stock per site. However, surviving colonies from the two apiaries were placed in one apiary in June 1991. Nearly all colonies were initially infested with V. jacobsoni, internal Acarapis, A. woodi (Rennie) and the external Acarapis, A. externus Morgenthaler and A. dorsalis Morgenthaler. To suppress these mite infestations, fluvalinate fumigation (0.0024 g) (Kulinčević et al. 1991) and menthol (50 g) treatments
were administered to all colonies before the experiment commenced. No subsequent treatments were employed after the initial chemical treatments.

All colonies were first inoculated with approximately 50 mites/inoculum in July 1990. Inoculation of mites was done by adding infested bees into the colonies. The inoculum was prepared by making a package of bees (about 10 lbs of bees) obtained from mixing worker bees from 3 heavily infested colonies. The number of colonies mixed depended on the levels of Varroa infestation in the colonies. The package of bees was sampled five times, one sample (approximately 50 bees/sample) in every corner of the box and one sample at the center, to derive the mean infestation rate. About 300 bees were estimated to harbor 50 mites. Bees in the package were sprayed with water to prevent them from flying. Inoculum bees were contained in screen wire cages (23 cm x 25 cm) which restricted the movement of bees but not the movement of mites and introduced between two brood frames. Inoculum bees were sampled and caged inside a screen tent to avoid additional introductions of mites into the colonies by lost or drifting bees. A second inoculation of 25 mites/inoculum was done in November 1990 using similar procedure. This trial was done from July 1990 to June 1992.
For trial 2, colonies were established using similar procedures. Two sites were used throughout the experimental period with 5 colonies per stock per site. All colonies received one mite inoculation of about 50 mites. These colonies were monitored for mite population trends from August 1991 to August 1992. For both trials, resistance to *V. jacobsoni* was evaluated on the basis of: 1) proportion of pupae infested with *Varroa* mites, 2) number of mites per infested pupa (including all mite life stages), 3) number of foundress *Varroa* per infested pupa, 4) number of progeny per foundress, 5) number of progeny per infested pupa, 6) proportion of infested pupae containing infertile females or foundresses with no progeny at the time of observation, and 7) levels of infestation on adult bees. The number of dead *Varroa* mites were also counted as an indication of grooming behavior by the different stocks.

The proportion of brood infested with *V. jacobsoni* was determined by examining 100 cells containing dark-eyed pupae with yellowish abdomens (approximately 16-17 days old) (Ifantidis 1984). Sampling was done every month. Levels of infestation of *Varroa* on adult bees were determined by sampling about 100-300 adult worker bees per colony every month. Adult bees were collected from a brood frame into a specimen jar. Bees were then placed on ice and immediately frozen upon arrival at the laboratory.
Adult bees were then washed with 70% ethyl alcohol. The number of mites and bees were counted to determine the levels of infestation on adult bees. The natural mortality of mites was monitored every month using bottom board traps sprayed with Pam (spray cooking oil). Longevity of the test queens were also recorded. Colonies were excluded from the experiment when queens changed by supersedure as well as when colonies became queenless.

Data for Varroa infestations inside the brood cell, Varroa infestations on adult bees, mite loads per infested pupa, numbers of foundresses per infested pupa, numbers of progeny per foundress, numbers of progeny per infested pupa, proportions of infested pupae with infertile foundresses, and numbers of dead mites were subjected to Analysis of Variance for repeated measures using the Proc Mixed procedure (SAS Institute, Inc. 1992). Before data analysis, percentages of brood infested and Varroa infestations on adult bees were transformed using the arc sine transformation. Means were considered not significantly different when confidence limits overlapped. Longevities of colonies were compared among stocks using the LIFETEST procedure (SAS Institute, Inc. 1990). Association of colony survival and the levels of Varroa infestation during the last month prior to death of queens or colonies was also tested using the same procedure. Pearson correlation analysis was used to test the
relationship between Varroa infestations, population sizes, and numbers of dead mites. A tolerance index was then calculated by multiplying months of survival with infestation levels of Varroa in the brood cells and analyzed using the General Linear Model procedure. Only dead colonies were included in this analysis.

Results

Population growth of Varroa jacobsoni as measured by brood infestation

For trial 1, a significant interaction between stock and sampling month was observed on the rates of infestation of Varroa on worker pupae (P< 0.0001)(Figure 2.1). Although levels of Varroa infestation in all stocks were low (less than 10%) from July 1990 to May 1991, a growth trend seemed to be emerging by November 1990. Varroa infestation in the Louisiana stock started to increase at this time with a significant increase observed in February. A sudden increase in the level of infestation in June 1991 was observed for all stocks with more increase in the Louisiana, ARS-Y-C-1 and Hastings stocks. F₁ hybrid colonies had the lowest infestation. This mean was significantly lower than the means of Louisiana and ARS-Y-C-1 stocks but not significantly lower than the mean of the Hastings stock. A similar trend was observed in July 1991. During the fall months (August to October 1991) comparably high levels of Varroa infestation
Figure 2.1. ANOVA and graph of the population growth of Varroa jacobsoni in four selected stocks of Apis mellifera for trial 1 (July 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
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% Infestation

Hastings  Louisiana  F₁ Hybrid  ARS-Y-C-1
were recorded in all the stocks. The last colony of the Louisiana stock died at the end of 1991. A distinct decrease in the infestation levels was observed during the winter months (January to March 1992). At this time only a few colonies were still alive: one colony for Hastings stock, two colonies for ARS-Y-C-1, three colonies representing the F₁ hybrid and no colony representative of Louisiana stock. In April and May, Hastings and F₁ colonies developed significantly higher levels of infestation than ARS-Y-C-1. *Varroa* infestation of the Hastings stock continued to increase until its last month of survival. There were two ARS-Y-C-1 colonies and one F₁ hybrid colony surviving at the end of the experiment in June 1992. No correlation between *Varroa* infestation and the amount of brood present inside the test colonies was detected.

For trial 2, no stock by month interaction (P< 0.0612) or stock differences were detected (P< 0.9260) (Figure 2.2). The growth of *Varroa* populations was faster in trial 2 than in trial 1. One month after the inoculation, levels of *Varroa* infestation already ranged from 5.8 to 8.9%. Infestation levels continued to increase rapidly. The stock by month data suggest that the infestation found in Hastings and F₁ colonies become more numerically pronounced through time, a condition which may have caused the weak stock by month interaction.
Figure 2.2. ANOVA and graph of the population growth of *Varroa jacobsoni* in four selected stocks of *Apis mellifera* for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
By June 1992, infestation levels significantly increased with the highest recorded in the hybrid colonies and Hastings stock. The ARS-Y-C-1 and Louisiana stocks followed behind in infestation rates. In July, there were three ARS-Y-C-1 colonies, one Hastings, two Louisiana and three hybrid colonies surviving. However, only one colony of the Louisiana stock and F₁ hybrid were alive by August. Rates of brood infestation of Varroa in trial 2 were not correlated with the amount of brood present inside the hives.

**Infestation levels of Varroa jacobsoni on adult bees**

The levels of Varroa infestation on adult bees for trial 1 varied significantly due to the interaction of bee genotypes and sampling month (P< 0.0001)(Figure 2.3). This means that differences among the stocks vary through time. Lowest infestations were recorded during the first ten months (August 1990 to May 1991) of mite inoculation. By June 1991, infestations of Varroa on adult bees of the ARS-Y-C-1 and the Louisiana stocks increased. Infestation on Louisiana adult bees increased to its maximum in July 1991, three months before the last colony of this stock died. High levels of infestation continued in the ARS-Y-C-1 stock until the end of the experiment when two ARS-Y-C-1 colonies survived. Varroa infestation of Hastings colonies drastically increased in September 1991. Low levels of infestation were consistently observed in the
Figure 2.3. ANOVA and graph of the percent infestation of Varroa jacobsoni on adult bees of four selected stocks of Apis mellifera for trial 1 (August 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
% Infestation

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- Hastings
- Louisiana
- F₁ Hybrid
- ARS-Y-C-1
Figure 2.4. ANOVA and graph of the percent infestation of *Varroa jacobsoni* on adult bees of four selected stocks of *Apis mellifera* for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly when confidence limits do not overlap (SAS Institute, Inc. 1992).
hybrid colonies up to September 1991. However, infestation of hybrids increased abruptly in October 1991. This infestation rate was comparable to that of Hastings and ARS-Y-C-1 stocks in October 1991. In January 1992, ARS-Y-C-1 attained their peak of infestation, which was comparable to that of the Hastings stock. The lowest January infestation was observed on the hybrid colonies. A distinct drop in Varroa infestations in all stocks was observed in February and April 1992. Infestation then increased attaining high levels by the end of the experiment in June 1992. No correlation was observed between adult bee population and infestation levels of Varroa on the adult bees.

In trial 2, Varroa infestation on adult bees ranged from 0.13 to 39% (Figure 2.4). Significant differences among the stocks were recorded (P< 0.0325). ARS-Y-C-1, Hastings and Hybrid stocks tended to have higher infestation of Varroa on adult bees than the Louisiana stock. No significant stock by sampling month interaction was observed (P< 0.2817). Adult bee population also was not correlated with Varroa parasitism on adult bees.

Natural mortality of Varroa jacobsoni

ANOVA revealed a significant interaction between stocks and month for trial 1 for the number of dead Varroa mites collected from the bottom board traps (P<0.0006)
(Figure 2.5). For the first six months, low numbers of dead mites were collected from all the stocks. In February 1991, numbers of dead mites gradually increased in all stocks. The highest numbers of mites were observed in the Louisiana stock in May and June 1991. Numbers of dead mites collected from the ARS-Y-C-1 stock colonies were also comparatively high in May 1991. Dead mite counts then dropped through the summer and winter and rose again.

In trial 2, there was a significant stock by sampling month interaction for the data concerning the number of dead mites collected (P< 0.0225)(Figure 2.6). The number of dead mites recovered from the bottom board traps was low for the first six months. In April, a distinct increase in mite mortality was observed for all the stocks with Hastings and ARS-Y-C-1 stocks reaching their maximum in June. During this month, the lowest count was observed in the Louisiana stock.

Significant correlations between infestation rates of Varroa in the brood cells and the numbers of dead mites were observed in the Hastings (r = 0.7424, n = 9, P< 0.0220) and hybrid colonies (r = 0.7107, n = 9, P< 0.0142) but not for Louisiana (r = 0.0539, n =9, P< 0.8991) and ARS-Y-C-1 stocks (r = -0.0522, n = 9, P< 0.8938).
Figure 2.5. ANOVA and graph of the number of dead Varroa jacobsoni collected from bottom board traps of four selected stocks of Apis mellifera for trial 1 (August 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars of the different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
No. of dead mites (x 100)

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Hastings
Louisiana
F₁ Hybrid
ARS-Y-C-1
Figure 2.6. ANOVA and graph of the number of dead *Varroa jacobsoni* collected from bottom board traps of four selected stocks of *Apis mellifera* for trial 2 (September 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
Number of mites per infested pupa

In trial 1, there were weak differences among the four genotypes (P< 0.0979) in the number of mites per infested pupa (Appendix B.1). These differences tended to be constant through time since the stock by month interaction was not significant (P< 0.9310). Infested pupa contained from 1 to 10 Varroa mites.

In trial 2, no differences between stock occurred in the number of mites per infested pupa (P< 0.2125) nor did the analysis indicate a stock by month interaction (P< 0.6859) (Appendix B.2). Regardless of the stock, 1 to 7 mites were found infesting a brood cell.

Other reproductive parameters

No differences were detected in the analyses of: number of foundress Varroa per infested pupa (1 to 3 foundresses) [Appendix B.3 (trial 1) and B.4 (trial 2)], number of progeny per foundress or reproductive rate (0 to 5 progeny) (Appendix B.5 and B.6 for trial 1 and trial 2, respectively), number of progeny per infested pupa (0 to 7 progeny) [Appendix B.7 (trial 1), B.8 (trial 2)] and proportion of infested pupae with infertile foundresses (0 to 47%) [Appendix B.9 (trial 1) and B.10 (trial 2)].

Queen or colony longevity

The survival of the queen or colony did not differ significantly among the four genotypes of honey bees (Log-rank $\chi^2 = 2.1039; \text{df} = 3; P< 0.5511$). Hastings stock
Figure 2.7. ANOVA and graph of the tolerance index of four stocks of *Apis mellifera* to *Varroa jacobsoni* using infestations in the brood cells. Bars with different letters indicate means are significantly different at $\alpha=0.05$ (LSMeans, SAS Institute, Inc. 1990).
survived mite infestations for 10.90±0.89 months, Louisiana stock 9.05±0.94, F₁ hybrid colonies 9.46±1.03 months and ARS-Y-C-1 11.04±1.22 months. However, a highly significant association between the infestation of Varroa in the brood cells and queen or colony mortality (Log-rank \(\chi^2 = 24.12; \text{df} = 1; \ P< 0.0001\)) was observed.

**Tolerance index of the four stocks of honey bees**

Analysis showed significant (\(P< 0.0236\)) differences in the tolerance index among the four stocks tested based on pupal infestation (Figure 2.7). Hastings had the highest tolerance level to Varroa mites followed by F₁ hybrid and ARS-Y-C-1 stocks. Louisiana stock was more sensitive to Varroa infestation having a lower tolerance index.

**Discussion**

Resistance and tolerance to parasitic mites are very difficult to measure because of many interacting factors. Using the created variable, tolerance index, we inferred from this study that Louisiana stock was more susceptible to Varroa infestation due to their tendency to die earlier with lower levels of Varroa infestation in the brood cells than Hastings, ARS-Y-C-1 and F₁ hybrid colonies.

The growth of Varroa populations in our test colonies varied through time. The increase in infestation recorded in February 1991 coincided with the decrease in
the amount of brood present inside the colonies. The onset of brood rearing in Florida occurred about mid-January. However, when brood rearing peaked in March and April 1991, the rates of Varroa infestation decreased. The reason for this fluctuation in mite infestation is unclear. Analysis showed that the percentage of infested brood was not correlated with the amount of brood present in the hives. However, the decrease in Varroa infestation was probably due to the presence of drone brood during these months. *V. jacobsoni* is known to prefer drone brood by a factor of 3.1-8.6 more than worker brood (Schulz 1984, Woyke 1987, Fuchs 1990). It is also possible that the proportion of uninfested brood increased during this period since the mite population does not increase at the same rate as brood rearing.

Generally, the highest Varroa infestations in brood cells were observed from spring to fall months with the lowest recorded during winter months. Our results showed that Hastings, ARS-Y-C-1 and the F₁ hybrid had higher levels of Varroa infestation inside the brood cells than the Louisiana stock. This observation corroborates the findings of Rosenthal et al. (1990) in Israel who found that A. m. *carnica* had higher infestation levels than A. m. *ligustica* colonies. However, in Germany Otten (1990) observed that these two subspecies (*carnica* and *ligustica*) had comparably lower infestations than A. m.
mellifera. A. m. carnica colonies in Brazil had higher infestation than the wild type Africanized bees but remained lower when compared to sister carnica colonies in Germany (Engels et al. 1986). Likewise, when Varroa infestation levels in A. m. ligustica were compared to Africanized bees (AHB), the ligustica type had higher infestation rates than AHB (Moretto et al. 1991). These authors also observed that infestation levels were higher in cooler regions of Brazil and thus, claimed that both climate and bee race affected seasonal populations of Varroa mites. It is possible that the reproductive ability of Varroa infestation in Hastings, ARS-Y-C-1 and Hybrid colonies may have been affected by temperature and other important environmental factors. Whether the genetic composition of Varroa mites played an important role needs further investigation. Varroa in Europe was thought to have been originated from Ussuria (Ruttner 1983) and Varroa mites in the U. S. are thought to be of South American origin (Delfinado-Baker & Houck 1989). Varroa was believed to have been introduced to South America from Japan (de Jong et al. 1982).

The reason for the increase in the infestation of Varroa in the Hastings, ARS-Y-C-1 and F₁ hybrid is unclear. These three stocks including the Louisiana stock, which had lower infestation levels, showed comparable numbers of mites per infested cell throughout
the study period. This observation may be an indication of similarity in the degree of attractiveness for Varroa reproduction. However, mite load tended to increase with the increase in the level of Varroa infestation inside the brood cells (more than 6 mites when infestation increased above 60%). A similar observation was reported by Moosebeckhofer et al. (1988). All our test stocks also showed similar numbers of foundress Varroa (1-3 females) inside infested pupae. Woo (1992) recorded a wider range of about 1-5 mites in worker brood of A. m. ligustica in Korea. Our results also showed comparable numbers of progeny (0-5) produced by one female mite. Woo (1992) observed about 5-6 progeny per female mite. However, Moosebeckhofer et al. (1988) reported 0.19 to 2.14 progeny per female Varroa in A. m. carnica in Germany, which is a smaller range than we observed.

Population growth of Varroa is affected by the number of reproductive females founding a cell. In Uruguay, colonies of racial hybrids of ligustica, iberica and carnica types maintained low levels of Varroa infestation due to the presence of high percentages of infertile females (60-90%) (Ruttner & Marx 1984). In the present study, we observed no differences in the proportion of infested cells containing infertile females among the test stocks which ranged from 0-100%. The highest proportion (100%) that we observed may be bias
since this phenomenon was recorded one month after the experiment began. Infestation rates at that period had an average of 0 to 1%. Otherwise, the proportion of infertile females ranged from 0 to 47%. This observation falls within the range reported by Ritter (1992) who observed 40-65% of the females were infertile in Tunisian colonies. In the Middle East, Ritter and de Jong (1984) observed 20-27% infertile females while in Brazil the authors found a higher percentage of 50-60%, a higher proportion than we typically observed in our evaluation.

Although Varroa reproduce inside the brood cell, this mite also parasitizes adult bees especially during periods with no available brood. According to Schulz (1984) and Woyke (1987), V. jacobsoni are phoretic on adult bees as they seek new hosts. The authors also observed that this mite species can survive outside brood cells for 10-13 days when suitable brood is not available. Isola (1987) claimed a longer survival of Varroa on adult bees; 40 days in spring and summer and up to 4 months during the winter months. The increased infestation in January 1992 (trial 1) may be due to the scarcity of brood during this month. Some colonies had small patches of brood while others had none during this winter month. It is also possible that a large population of mites had already been established at this time and thus, a high proportion of invading phoretic mites was therefore
present. Adult samples were usually collected from brood frames containing old larvae.

In two sampling months (trial 1), the numbers of dead Varroa mites collected from bottom board traps of the Louisiana stock were highest among the four stocks. This observation may be an indication of elevated grooming behavior of this stock or may also be due to other stock characteristics, which caused the early death of Varroa mites. Nonetheless, this characteristic did not influence the ability of this stock to tolerate Varroa infestations. For trial 2, higher numbers of dead mites were recorded in the Hastings, hybrid and ARS-Y-C-1 stocks. Yet, these stocks maintained higher levels of Varroa infestation in the brood cells and on adult bees. Further experiments on the grooming behavior of these stocks should be done to clarify this matter.

Overall, Hastings and ARS-Y-C-1 stocks demonstrated moderate tolerance to V. jacobsoni based on the stocks tendency to live longer with higher proportions of pupae infested with mites. Louisiana stock had lower infestations possibly due to a better grooming behavior. Nonetheless, the Louisiana stock is more susceptible since it died earlier with lower levels of Varroa infestation than the three stocks.
References


CHAPTER III

POSTCAPPING DURATION OF FOUR STOCKS OF APIS MELLIFERA L. AND RESULTS ON THE SURVIVAL AND DEVELOPMENT OF INOCULATED DEUTONYMPS OF VARROA JACOBSONI OUDEMANS (ACARI: VARROIDAE)
Introduction

Varroa jacobsoni Oudemans reproduces only inside the capped cells containing honey bee (Apis sp.) prepupae. Therefore, the capped duration is a crucial element in the reproductive success of Varroa mites. The duration of this stage influences both the total number of progeny produced and, most importantly, the number of daughter females that reach adulthood.

Honey bees with a shorter capped period will support the production of fewer reproductive females (Moritz & Hanel 1984, Woyke 1987, Buchler & Drescher 1990). Moritz (1985) observed that African honey bees (A. mellifera capensis Escholtz and A. m. scutellata) have capped durations of 9.7 and 11.2 days, respectively. Both are shorter than that of the European honey bee A. m. carnica Pollman (12.1 days). This contrast suggests that African subspecies of bees support a slower growth of mite populations. This at least is the case for A. m. capensis studies in Germany (Moritz & Hanel 1984). However, whether or not the immatures die or seek a new host and survive has not been studied. It is generally thought that immature female mites die when they are released from the cell upon emergence of the parasitized honey bee.

The objectives of this study were to determine the duration of the capped stage of four stocks of A. mellifera and to explore the survival and development of
deutonymphal stage of Varroa mites when inoculated in newly capped larvae.

Materials and Methods

Postcapping duration of four selected stocks of Apis mellifera

Four stocks of honey bees, A. mellifera were used in this study: Hastings stock (from A. m. carnica origins) from Northern Saskatchewan, ARS-Y-C-1 stock from Yugoslavia (from A. m. carnica origins), F1 hybrids between ARS-Y-C-1 x Hastings stocks and general Louisiana stock. All queens were reared by grafting and each was instrumentally inseminated with 8 μl of semen. For the ARS-Y-C-1, semen was from drones representing all 15 ARS-Y-C-1 sublines, pooled in equal proportions and mixed prior to insemination.

Grafting (the transfer of larvae from one cell to another usually practiced in queen production) was used as described by Harbo (1992) with minor modifications. Prior to grafting, two colonies were established as host colonies or colonies that received the grafted larvae. A total of five trials (representing five colonies from each stock) were conducted; four trials in host colony 1 and one trial in host colony 2. Trials conducted in host colony 1 were at least two weeks interval with the exception of 4 and 5 which were grafted side by side into the brood frame.
In each test colony, an empty frame was inserted at the middle of the brood nest to provide a place for the queen to lay. Frames were inspected the next day for the presence of eggs, which were used in grafting the first day after the eggs hatched. At that time, a brood frame with young larvae from each host colony was chosen to receive the grafted larvae. Colonies were fed with sugar syrup and pollen patties from one week prior to grafting to until the grafted larvae were capped by the host colony's bees.

At the center of a frame that received the grafted larvae, an area occupying 8 rows x 20 cells was established. Two rows were assigned randomly for each stock per trial. Larvae inside the cells within the area were discarded and cells were cleaned using a grafting needle. For better larval acceptance and survival, a drop of royal jelly was placed at the bottom of each cell.

In order to determine the duration of the capped period, grafted larvae were inspected for sealed larvae every two hours starting on the 4th day after grafting. Sealed larvae were then mapped on a sheet of transparency. Nine days after capping, pupae were number-tagged (different color for each stock) and then placed in an incubator set at 34.5°C for emergence. Emergence of bees was observed every two hours. This investigation was conducted between August 27 to October 4, 1992.
Data were analyzed using the Randomized Block Design with replications as blocks and stocks as treatments using the General Linear Model Procedure (SAS Institute, Inc. 1990).

Survival and development of deutonymphs of Varroa inoculated in newly capped larvae.

This portion of the study was conducted in Florida well before Varroa was detected in Louisiana in August 1992. The test colony (5-frame nucleus) of open mated commercial stock was established at the Baton Rouge Laboratory to obtain a Varroa mite-free colony. This claim was substantiated by examining about 50 worker pupae for the presence of mites and finding none. The colony contained three brood frames, two of which had mostly old larvae (L5) about to be sealed, and two honey frames. Inoculum nymphs of Varroa were collected from a Varroa-infested colony using dark-eyed pupae (approximately 16-17 days old) or pupae with yellowish abdomens. Nymphal stages used were both the mobile and immobile stages of the deutonymph as described by Ifantidis (1983).

Deutonymphs were transferred to cells that contained larvae, which had recently been sealed. Such larvae provided the best chance for survival of the transferred deutonymphs. About 40 newly capped larval cells were inoculated by slightly opening the cappings of the brood cells using the tip of a forcep. Each larval
cell received one mite. Thereafter, cappings were repositioned. As a control, cappings of newly capped larvae were opened and repositioned without mite inoculation.

The location of mite-inoculated larvae and the control cells in the comb were mapped on a sheet of transparency. Brood comb was then placed back in the host colony. After one week, all accepted cells were opened and examined for mite survival and development.

Results

Postcapping duration of four stocks of *A. mellifera*

Analysis showed that stocks significantly differ (P< 0.0001) in the duration of their capped period (Figure 3.1). ARS-Y-C-1 had the longest capped period with the shortest duration observed in the F₁ hybrids. Hastings and Louisiana stocks had comparable capped periods.

Significant differences (P< 0.0001) in the postcapping duration among the trials were also detected (Figure 3.1). Replication two, which was conducted in a host colony different from the host colony used for the other four replications, was comparable to the mean observed in replication one. The two replications (4 and 5) that were simultaneously conducted had significantly (P< 0.0198) different capped periods. The shortest duration was observed in replication four.
Figure 3.1. ANOVA and graph of the average duration (h) of capped period of four stocks of *Apis mellifera* and average duration of the five trials conducted from August 27 to October 4, 1992. Means with different letters are significantly different at $\alpha=0.05$ (LSMeans, SAS Institute, Inc. 1990).
Table 3.1. Survival and development of *Varroa jacobsoni* deutonymphs inoculated in newly capped larvae of *Apis mellifera*.

<table>
<thead>
<tr>
<th></th>
<th># cells inoculated</th>
<th># cells accepted</th>
<th># cells with living mites</th>
<th># cells with dead mites</th>
<th># cells without mites</th>
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</thead>
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<tr>
<td><strong>Capped brood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>immobile stage</td>
<td>28</td>
<td>21</td>
<td>4(1)</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>mobile stage</td>
<td>12</td>
<td>11</td>
<td>1(1)</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><strong>Control (no mite inoculation)</strong></td>
<td>80</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers inside () indicate the number of living deutonymphs.
Survival and development of nymphal stages of Varroa inoculated in newly capped larvae.

Table 3.1 shows the survival and development of deutonymphs inoculated in newly capped larvae. Among the 32 surviving pupae which were inoculated with Varroa, a few mites survived. However, no significant differences were observed on the survival of the two nymphal stages of Varroa ($\chi^2 = 3.957; \text{df} = 2; P< 0.138$). Using the immobile stage as inoculum, five mites survived, one of which was still in the nymphal stage and four of which had attained maturity. Only two mobile deutonymphs were able to survive; one reached adulthood and one showed development but still remained a nymph (immobile stage). Some mites did not develop and died inside the brood cell; others were not found at the final examination.

Discussion

There is great variability in the duration of the postcapping periods of different subspecies of A. mellifera. In the African honey bees (A. m. scutellata and A. m. capensis), the capped period is 22 to 58 h less than A. m. carnica which has a mean of 290.4 h (Moritz 1985). This value for the A. m. carnica type is 1.22 and 5.03 h longer than the period observed in the A. m. carnica bees imported from Yugoslavia (ARS-Y-C-1) and from Northern Canada (Hastings), respectively. Otten (1990) observed a mean duration of 288.2 h for an additional
stock of _A. m. carnica_ which is about the average of the two _A. m. carnica_ stocks in this study. Otten also recorded a mean of 287.7 h for an _A. m. ligustica_ strain; about 2.7 h more than the Louisiana stock used in this study. Le Conte and Cornuet (1989) observed an even longer period for _A. m. ligustica_ of 294.24 h, which they claimed had been affected by larval feeding. By using 26 test colonies, Harbo (1992) found the average capped period to be 285.4 h. F₁ hybrids of our test stocks had an average postcapping duration which was 3.11 h shorter than what Harbo observed. Perhaps heterosis typically reduces the postcapping duration as suggested by Le Conte and Cornuet (1989). The longest capped duration was observed in the ARS-Y-C-1 (_A. m. carnica_) stock. However, this duration is shorter than that reported by Moritz (1985) and may actually reflect the selection for resistance to _Varroa_ in the stock's history (Kulinfievic & Rinderer 1988, Kulinfievic et al. 1992).

Environment also affects the postcapping period. By studying the capped period in three separate years and different months in a single stock of bees, Buchler and Drescher (1990) found seasonal differences in the duration of the capped stage in Germany. They estimated that during early summer, the capped period is 7 h shorter than in late summer. Seasonal variation in the capped duration was also reported by Schousboe (1990). Le Conte and...
Cornuet (1989) suggested that the capped period is affected by larval feeding of the rearing colonies. Our study contributes further information on sources of environmentally induced variation. The capped period varied significantly within one month of observation. This difference was twice the seasonal differential reported by Buchler and Drescher (1990). Additionally, two trials conducted simultaneously in the same colony differed by a significant 4 h. This discrepancy most likely was not generated by differential feeding by the nurse bees. More likely causative factors are slight temperature differences in different areas of the hive as suggested by Buchler and Drescher (1990). Similar observations were also reported by Harbo (1992). In his study, larvae kept in an incubator in nutritional poverty for about 8.5 h did not show an altered capped period duration but rather had a reduced survival rate and a longer uncapped period. Clearly, environmental influences profoundly affect the postcapping period. Such influences vary in both short and longer (seasonal) time frames. Postcapping period evaluations will be improved when these sources of variation are understood and controlled.

The duration of the capped period has been thought to be the crucial determinant of the number of matured female mites at bee emergence (Otten 1990). As in A. m.
capensis, production of sexually matured female progeny is greatly reduced in bees having shorter capped periods (Moritz & Hanel 1984). Buchler and Drescher (1990) indicated an 8.7% decline in the growth of Varroa populations with a 1-h reduction in the capped period of honey bees. In addition, all immatures are believed to die after bee emergence.

The survival of inoculated deutonymphs of Varroa showed the possibility that mobile stage deutonymphs may seek and find a new host upon the emergence of the bee. A brood cell that is about to be capped (with a small hole remaining as entrance) may provide such an opportunity. Immobile deutonymphs may even, on rare occasion, fall from emerged bees into an appropriate host cell, survive and develop to adulthood. Adults derived from these circumstances are however, unmated because of the absence of males in the second host cell where they complete their development. There are many reports of infertile females (Ritter & De Jong 1984, Ruttner & Marx 1984, Otten 1990, Ritter et al. 1990) and cases where only male progeny are present in the cell with the reproductive female. Perhaps at least some of the instances can be explained by the final development of deutonymphs in second cells that do not contain males with which to mate.
References


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CHAPTER IV

COMPARATIVE RESISTANCE OF FOUR STOCKS OF APIS MELLIFERA L. TO INFESTATIONS BY ACARAPIS DORSALIS MORGENTHALER, ACARAPIS EXTERNUS MORGENTHALER AND ACARAPIS WOODI (RENNIE) (ACARI: TARSONEMIDAE)
Introduction

There are three *Acarapis* species known to be specific to *Apis mellifera* L. These include the internal *Acarapis*, *A. woodi* (Rennie), which lives and reproduces in the prothoracic tracheae of a bee host, and the two external *Acarapis*, *A. dorsalis* Morgenthaler which uses the scuto-scutellar groove of the thorax, and *A. externus* Morgenthaler, which is found mostly on the venter of the neck and in the tentorial pits. All species use the wing axillaries for migration. External *Acarapis* sometimes use this area for reproduction (Royce et al. 1988).

The tracheal mite, *A. woodi*, is the most studied *Acarapis* because of its reported damage on infested colonies. It is known to shorten the longevity of infested adult bees (Bailey 1958, Maki et al. 1988, Royce & Rossignol 1990). However, no decreased longevity was observed by Gary and Page (1989). High winter losses have been reported to be associated with high levels of infestations (Eischen 1987, Eischen et al. 1989, Furgala et al. 1989).

The two external *Acarapis*, *A. dorsalis* and *A. externus*, are largely ignored because they are considered harmless to honey bees. Nonetheless, the economic impact of external *Acarapis* has not been studied and may be greater than perceived. In Oregon, these three species were observed to coexist in a single colony (Burgett et
al. 1989). However, individual worker-bee hosts are rarely parasitized by more than one *Acarapis* species. This phenomenon may be explained as adaptation to avoid food competition. All three species are known to be hemolymph feeders (Örösi-Pal 1934).

The present study was conducted to monitor trends of natural *Acarapis* populations in four stocks of honey bees infested with *V. jacobsoni* as well as to assess resistance of the stocks to *Acarapis* mites.

**Materials and Methods**

With the wide distribution of the four parasitic mites of honey bees in the U.S., exclusive maintenance of one mite species for experimental purposes is impossible. Therefore, in our colonies used primarily for Varroa mite research, the seasonal trends of *A. dorsalis*, *A. externus* and *A. woodi* infestations were also monitored. Four selected stocks of *A. mellifera* were used: ARS-Y-C-1 (originating from *A. m. carnica* from Yugoslavia), Hastings (originating from *A. m. carnica* from Northern Saskatchewan), *F₁* hybrids between ARS-Y-C-1 and Hastings stocks, and general commercial Louisiana stock. Queens from each stock were instrumentally inseminated with 8µl of semen of the appropriate type and established in two-super Langstroth hives. Two trials were conducted. Before the study commenced, all colonies for the first trial were treated with fluvalinate (0.0024 g) and 50 g
menthol per colony to control Varroa and tracheal mites, respectively. Colonies for the second trial were likewise treated with fluvalinate before the experiment commenced to lessen Varroa mite population but no menthol treatment was applied. Thereafter, no treatment to control mites was applied. The first trial was done from July 1990 to June 1992 and the second trial from August 1991 to August 1992. Both trials were conducted in Northern Florida.

Test colonies were not inoculated with Acarapis mites. Instead, initial Acarapis infestations of all colonies were determined by examining 30 bees per colony. The presence of different developmental stages of the three Acarapis species was determined under a dissecting microscope. These species are morphologically similar and thus, difficult to identify. Therefore, differentiation was based on their location on the honey bee hosts. Mites on the scuto-scutellar groove were identified as A. dorsalis; A. externus were mites found on the neck and tentorial pits; and mites inside the prothoracic tracheal trunks were A. woodi. Bee samples for Acarapis examination were taken from samples intended for Varroa detection using the wash method. Before washing the bees, 50 bees were individually inspected for Varroa and frozen until examination. Out of this sample, thirty bees were individually inspected for the presence of the three Acarapis mites. Wing axillaries were not examined for
migrating mites. Examination of bees for tracheal mites was done using the thoracic dissection (Lorenzen & Gary 1986). The proportion of adult workers infested was determined and number of mites per infested bee was counted. Sampling was done every two months.

Data on the proportion of bees infested and number of mites per infested bee through time were subjected to Analysis of Variance for repeated measures using the Mixed Procedure (SAS Institute, Inc. 1992). Before analysis, data for the proportion of bees infested were transformed using the arc sine transformation to make variances homogeneous. Means were considered significantly different when confidence limits did not overlap. The LIFETEST procedure was used to analyze queen or colony survival and its association with Acarapis infestations during the last month of survival. Initial Acarapis infestations and infestations during the last month of survival were analyzed as a Randomized Block Design using General Linear Model Procedure. Means were compared using LSMeans; mean differences were considered significant at α=0.05 (SAS Institute, Inc. 1990).

Results

Seasonal population fluctuation

A) Acarapis dorsalis

There was a significant (P< 0.0281) stock by sampling month interaction on the levels of A. dorsalis
infestation (trial 1). Before test queens were introduced into the colonies in June 1990, comparably (P< 0.8263) low levels of *A. dorsalis* infestation of the original colonies were observed (Figure 4.1). In August 1990, a distinct drop in *A. dorsalis* population was recorded. This decrease was probably due to the chemicals applied in June 1990. The growth of the *A. dorsalis* populations in the Hastings stock apparently was faster than in any of the test stocks. A clear trend was observed in both years of observation. Infestations on Hastings stock started to increase in October with the highest infestation recorded in December and February. Lowest infestation was observed in August. In the F₁ hybrid, ARS-Y-C-1 and Louisiana stocks, *A. dorsalis* infestations remained well below 5% infestation levels throughout the study.

A significant (P< 0.0383) interaction between stock and sampling month for trial 2 was also detected. The same trend was recorded (Figure 4.2). Hastings stocks had the highest infestations throughout the study with a peak observed in October 1991. Similarly, the three other stocks maintained less than 5% levels of *A. dorsalis* infestation throughout the period of study.

B) *Acarapis externus*

A significant (P< 0.0004) interaction between bee stocks and sampling month was observed on the proportion of bees infested with *A. externus* (trial 1). The initial
Figure 4.1. ANOVA and graph of the proportion of bees infested with *Acarapis dorsalis* for trial 1 (June 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
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<tr>
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<tr>
<td>Stock x Month</td>
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<td>0.0281</td>
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</tbody>
</table>

% Infestation

- Hastings
- Louisiana
- F_1 Hybrid
- ARS-Y-C-1
Figure 4.2. ANOVA and graph of the proportion of bees infested with *Acarapis dorsalis* for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
Infestation of this mite species did not (P< 0.9832) differ significantly among the stocks which ranged from 11±5 to 14±5% (Mean±SE) (Figure 4.3). For the first year of evaluation, infestation of A. externus was maintained at comparably low levels (below 10%) on all the stocks. In August 1991, infestations gradually increased in the hybrid colonies and Louisiana stocks. All stocks increased in infestations in October before the last colony representing Louisiana stock died. Peaks of A. externus infestations were observed in December for the F₁ hybrid and ARS-Y-C-1 stocks. A distinct decrease in infestation levels was observed in February in the survivor colonies and remained low until the end of the experiment. A. externus in the Hastings stock maintained the lowest infestation (about 2%) throughout the experimental period except in October 1991.

In trial 2, no (P< 0.3478) significant interaction between stock and month was detected (Figure 4.4). Stock effect also showed no (P< 0.9246) significant differences.

C) Acarapis woodi

In trial 1, a highly significant (P< 0.0001) interaction between stock and sampling month was observed. When the experiment was initiated, A. woodi infestations in all the stocks were similarly (P< 0.5223) high ranging from 20.6±6.06 to 32.39±6.93%. There was an apparent decline in infestation in August 1990 due to the menthol
Figure 4.3. ANOVA and graph of the proportion of bees infested with *Acarapis externus* for trial 1 (June 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
### ANOVA Table

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<td>2.31</td>
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### Graph

- **Y-axis:** % Infestation
- **X-axis:** Months (1990-1992)
- **Legend:**
  - Hastings
  - Louisiana
  - F₁ Hybrid
  - ARS-Y-C-1

*Note: Initial infestation is indicated by an asterisk.*
Figure 4.4. ANOVA and graph of the proportion of bees infested with *Acarapis externus* for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
and fluvalinate treatments administered in June. Thereafter, tracheal mite infestations in the hybrid colonies continued to decrease and were maintained at low levels throughout the experimental period (Figure 4.5). Likewise, ARS-Y-C-1 stock never reestablished their initial infestation levels. In February 1991, infestation in the Louisiana and Hastings stocks started to increase reaching a maximum in October, just before the death of the last Louisiana colonies. For the Hastings stock, there was a drop in the level of infestation during December, a recovery to a high level in February and an abrupt decline in April until the last colony died in June.

In trial 2, the interaction between the stock and sampling month was also highly significant ($P < 0.0001$) (Figure 4.6). The initial infestation of *A. woodi* was significantly higher in the Louisiana stock at the start of the experiment as compared to less than 10% in the three other stocks. Tracheal mite infestation remained significantly higher in this stock with its peak recorded in October, one month after the experiment started. Infestation then started to decline with a distinct drop in April until the end of the experiment. *A. woodi* infestation in the Hastings stocks increased gradually with the highest infestation observed in February and decreased until the end of the experiment. As in trial 1,
Figure 4.5. ANOVA and graph of the proportion of bees infested with *Acarapis woodi* for trial 1 (June 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
% Infestation

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<td>Stock x Month</td>
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</table>

Hastings Louisiana F<sub>1</sub> Hybrid ARS-Y-C-1
Figure 4.6. ANOVA and graph of the proportion of bees infested with Acarapis woodi for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
ARS-Y-C-1 and the F₁ hybrids maintained less than 15% infestation rates throughout the experimental period.

**Number of mites per infested worker bee**

The number of *Acarapis* mites per infested bee was monitored from August 1990 to August 1991 for trial 1 only. No (P< 0.1397) significant interaction and no (P<0.1558) significant differences in the number of *A. dorsalis* per infested bee was observed among the stocks (Figure 4.7). Likewise, initial infestation of this mite species did not (P< 0.3824) differ among the stocks. Worker bees from any stock infested with this mite species sustained about 1-3 mites through time.

For *A. externus*, significant interaction between stock and sampling month was detected (P< 0.0003) (Figure 4.8). The initial number of *A. externus* per infested bee did not (P< 0.2719) differ significantly among the stocks which ranged from 2.59±0.35% to 3.47±0.31%. For the first ten months (until April), mite load in all the test stocks was limited to about one mite only. However, a sharp increase to about 2-3 mites in June and August was observed in the F₁ hybrid colonies.

For the number of *A. woodi* per infested bee, a significant (P< 0.0069) interaction between the stocks and sampling month was observed (Figure 4.9). The density of tracheal mites in all the stocks before the experiment began did not (P< 0.7061) differ ranging from 8.76±1.8 to
Figure 4.7. ANOVA and graph of the number of *Acarapis dorsalis* per infested bee (June 1990 to August 1991). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
Figure 4.8. ANOVA and graph of the number of *Acarapis externus* per infested bee (June 1990 to August 1991). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
Figure 4.9. ANOVA and graph of the number of *Acarapis woodi* per infested bee (June 1990 to August 1991). Unlabeled groups of stocks within months do not differ significantly. Bars with different letters indicate means are significantly different when confidence limits do not overlap (SAS Institute, Inc. 1992).
11.37±2.06 mites. Mite load in all stocks gradually decreased probably because of the menthol and fluvalinate applications with a minimum recorded in October. In December, mite load steadily increased with a distinct peak observed in June for both Hastings and Louisiana stocks. Host bees from these stocks had about 13 to 18 mites. Mite load decreased again in August. Infested bees from the F₁ hybrid and ARS-Y-C-1 colonies had fewer numbers of tracheal mites.

**Levels of Acarapis infestation during the last month of queen or colony survival and their association with colony survival**

Table 4.1 presents the infestation levels of the three *Acarapis* species in the four test stocks during their last month of queen or colony survival. Results showed no significant differences in the levels of *A. dorsalis* (P< 0.0748) and *A. externus* infestation (P< 0.0739) before all colonies died regardless of stock. *A. woodi* infestation differed significantly (P<0.0347) among the stocks. Hastings and Louisiana stocks had the highest infestation rates. Lowest infestations were observed in the F₁ hybrid and ARS-Y-C-1 stocks. Longevity of the queens or colonies did not differ significantly among the stocks [Log-rank $\chi^2 = 2.10$; df = 3, P< 0.5511 (trial 1)]. Results showed that colony longevity was not associated with *Acarapis* infestations
Table 4.1. Infestation levels of *Acarapis* species during the last month of queen or colony survival and longevity of four selected stocks of *Apis mellifera*.

<table>
<thead>
<tr>
<th>Bee stocks</th>
<th>% <em>A. dorsalis</em>¹</th>
<th>% <em>A. externus</em>²</th>
<th>% <em>A. woodi</em>³</th>
<th>Longevity⁴</th>
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</thead>
<tbody>
<tr>
<td>Hastings</td>
<td>6.90±1.32</td>
<td>3.78±3.20</td>
<td>27.64±5.02ᵃ</td>
<td>10.90±0.89</td>
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<tr>
<td>Louisiana</td>
<td>4.26±1.42</td>
<td>12.18±3.46</td>
<td>33.55±5.41ᵃ</td>
<td>9.05±0.94</td>
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<tr>
<td>F₁ Hybrid</td>
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<td>3.02±4.58ᵇ</td>
<td>9.46±1.04</td>
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<tr>
<td>ARS-Y-C-1</td>
<td>1.18±1.26</td>
<td>7.74±3.06</td>
<td>2.55±4.8ᵇ</td>
<td>11.04±1.22</td>
</tr>
</tbody>
</table>

¹not significant, (P< 0.0748)
²not significant, (P< 0.0739)
³significant, (P< 0.0347)
⁴not significant, log-rank χ² = 2.10; df = 3; P< 0.551

n = 90 colonies
[Log-rank $\chi^2 = 0.0003$, df = 3, $P < 0.9868$ (A. dorsalis);
Log-rank $\chi^2 = 0.8072$, df = 2, $P < 0.3690$ (A. externus);
Log-rank $\chi^2 = 0.7290$, df = 2, $P < 0.3932$ (A. woodi)].

Discussion

Based on field evaluation (two years for trial 1 and one year for trial 2), ARS-Y-C-1 and the F$_1$ hybrid consistently displayed considerable resistance to A. woodi parasitism. Infestations in these stocks were maintained to about 10% throughout the experimental periods. This observation was also confirmed by the results of our bioassay (choice experiment discussed in Chapter V). Since Hastings stock did not show any level of resistance beyond that of the ARS-Y-C-1 stock, the resistance observed in the F$_1$ hybrid indicates that the genes conferring resistance to ARS-Y-C-1 and their hybrid may be dominant. Reduced infestations of tracheal mites had also been observed in the Buckfast stock both in the U. S. and English type (Milne et al. 1991, Lin et al. 1992). Levels of A. woodi infestation above 25% are believed to cause economic damage in bee colonies (Eischen et al. 1989, Otis & Scott-Dupree 1992). Since our results showed about 10% infestations in the ARS-Y-C-1 and F$_1$ hybrid, this suggests that colonies of these stocks will require little or no treatment for tracheal mite control. In addition, these stocks including Louisiana stock were resistant to A. dorsalis infestation. However, infestations of A.
externus on these three stocks were relatively high on two occasions (trial 1). Whether or not this is a clear indication of susceptibility of these stocks to A. externus needs further study since the results for trial 1 were not consistent with trial 2. Louisiana stock was resistant to A. dorsalis but susceptible to A. woodi infestation. The sudden decrease in the tracheal mite infestations in this stock in trial 2 was probably due to the death of the highly infested colonies, thus lowering the average infestation rate. The Hastings stock was resistant to A. externus but susceptible to A. woodi and A. dorsalis. Infestation by tracheal mite in this stock was consistently higher than it was in the resistant stocks in both trials.

Because of the ubiquity of these three Acarapis species, multiple infestation of a colony is usually seen. However, consequences of the concurrent parasitism of the three Acarapis species in honey bee colonies has not been studied. When the two external Acarapis are present, A. dorsalis seemed to be a more dominating external species than A. externus (Burgett et al. 1989, Ibay 1989, de Guzman & Burgett 1990, Bailey & Ball 1991). Similar results were obtained in the present study. A. dorsalis was observed more often than A. externus. However, infestation levels of A. externus are usually higher than A. dorsalis.
Overall, *A. woodi* seemed to be the dominating species among the three species both at colony and individual host bee levels. This may be attributed to the ability of this *Acarapis* species to reproduce faster as indicated by the higher number of mites observed inside the trachea. This ability may also explain the virulence of *A. woodi* in honey bees as compared to the two external *Acarapis*. More mites per infested bee will mean more feeding sites which are instrumental in the occurrence of secondary infections.
References


CHAPTER V

TRACHEAL MITE, ACARAPIS WOODI (RENNIE) (ACARI: TARSONEMIDAE) RESISTANCE OF SELECTED STOCKS OF HONEY BEES AND TWO OF THEIR HYBRIDS
Introduction

The parasitism of the two major parasitic mites \textit{Acarapis woodi} (Rennie) and \textit{Varroa jacobsoni} Oudemans] in honey bee colonies has caused frustrations and economic hardships to the American beekeeping industry. Despite tremendous efforts to control these parasites using chemicals, colony losses continue to increase across the United States.

The use of honey bee stocks resistant to parasitic mites as an alternative mite control is increasingly recognized as a long term solution with good potential. Adams (1987) developed the Buckfast stock in Great Britain and claimed that this stock was resistant to tracheal mites. This stock was later brought into the U. S. for commercial purposes. Using this stock, studies conducted in the U. S. and Canada have confirmed its resistance to tracheal mites (Milne et al. 1991, Lin et al. 1992).

Another stock of honey bees that has been reported to be resistant to parasitic mites is an \textit{Apis mellifera carnica} Pollman stock from Yugoslavia (Kulinčević & Rinderer 1988, Kulinčević et al. 1992). This stock was imported into the U. S. in 1989 for further study (de Guzman et al. 1990, Rinderer et al. 1993). Recently, Rinderer et al. (1993) showed that this stock (ARS-Y-C-1) has considerable resistance to tracheal mites.
The present study was conducted to compare the resistance of these two stocks and their reciprocal hybrids to A. woodi using a choice experiment or bioassay. If the stocks differed in the genetic elements underlying their resistance, perhaps the resistance of hybrid colonies would be further enhanced.

Materials and Methods

Five groups of A. mellifera were evaluated for their resistance to tracheal mites, A. woodi. These stocks include: (1) ARS-Y-C-1 [A. m. carnica originated from Yugoslavia, (2) Buckfast stock (imported from United Kingdom), (3) ARS-Y-C-1 queen x Buckfast drones, (4) Buckfast queen x ARS-Y-C-1 drones, (5) Louisiana stock available at the Baton Rouge Bee Laboratory. Stocks 1 and 2 served as potential controls to evaluate the F₁ crosses (3 and 4). Stock 5 served as an additional control to establish the comparative resistance of stocks 1 and 2.

Queens were individually inseminated with 4μl of semen and established in two-super colonies located at two different sites. The method used to evaluate resistance to tracheal mites was as described by Gary and Page (1987). About one to two frames of emerging brood were removed from each colony, placed separately in nylon mesh, and reared in an incubator maintained at 34.5°C for bee emergence. Approximately 150 young bees (>24 h-old) from each colony were individually marked on the abdomen using
enamel paint. Marked bees from each colony were placed in separate cages inside an incubator until all bees were marked. Thereafter, marked bees were simultaneously introduced into an infested colony. Honey bees from each site utilized a separate host colony. The host colony for site 1 colonies had an infestation level of 32% during the first trial and increased to 47% during the second trial. For site 2 colonies, the host colony had 27% and 83% for the first and second trials, respectively.

After 72 h, at least 30 marked bees were collected and examined for tracheal mite infestation. All marked bees present in the colony were collected after 14 days. At this time, at least 50 bees per colony were dissected using the thoracic dissection (Lorenzen & Gary 1986). Number of mites per infested bee was counted. Wing axillaries were also checked for migrating mites. Data were analyzed as a split plot in Completely Randomized Design. Means were compared using the LSMeans method (SAS Institute, Inc. 1990). Differences were considered significant at α=0.05.

Results

A significant (P< 0.0001) stock effect was observed on the proportion of bees infested with A. woodi in the tracheae alone (Figure 5.1). Louisiana stock had the highest level of tracheal mite infestation. ARS-Y-C-1 bees had the lowest infestation rate which was comparable
Figure 5.1. ANOVA and graph of the proportion of bees infested with *Acarapis woodi* inside the prothoracic tracheae of five stocks of honey bees. Bars with different letters indicate means are significantly different at $\alpha = 0.05$ (LSMeans, SAS Institute, Inc. 1990).
Figure 5.2. ANOVA and graph of the proportion of bees infested with *Acarapis woodi* inside the prothoracic tracheae plus wing axillaries of five stocks of honey bees. Bars with different letters indicate means are significantly different at $\alpha = 0.05$ (LSMeans, SAS Institute, Inc. 1990).
to that of Buckfast, ARS-Y-C-1 x Buckfast and Buckfast x ARS-Y-C-1 colonies. Infestation of the trachea was significantly \( (P < 0.0275) \) higher after 14 days than 3 days of exposure to the host colonies.

Total infestations (tracheal + wing axillaries infestations) also showed significant differences \( (P < 0.0001) \) and followed the same trends found for tracheal infestations alone (Figure 5.2). Louisiana stock significantly maintained the highest infestation with the lowest observed in the ARS-Y-C-1 stock, the Buckfast stock and the reciprocal crosses, a group which did not vary significantly. No differences \( (P < 0.8843) \) were observed for the total infestation when comparing during 3 and 14 days of exposure.

The proportion of infested bees having one trachea infested (unilateral infestation) differed significantly \( (P < 0.0001) \) (Figure 5.3). Louisiana stocks significantly had the highest unilateral infestation. More unilateral infestation was observed 14 days after exposure than 3 days post-inoculation. However, the difference was not strong \( (P < 0.0696) \). Bilateral infestation also displayed significant differences \( (P < 0.0001) \) following similar trend (Figure 5.4). Bilateral infestation tended to become higher as the bees were exposed longer (14 days) than after 3 days. The difference was also weak \( (P < 0.0748) \).
Figure 5.3. ANOVA and graph of the proportion of bees with unilateral infestations of *Acarapis woodi* in five stocks of honey bees. Bars with different letters indicate means are significantly different at $\alpha = 0.05$ (LSMeans, SAS Institute, Inc. 1990).
Figure 5.4. ANOVA and graph of the proportion of bees with bilateral infestations of *Acarapis woodi* in five stocks of honey bees. Bars with different letters indicate means are significantly different at $a = 0.05$ (LSMeans, SAS Institute, Inc. 1990).
All stocks showed a similar number of tracheal mites per infested bee ranging from 5.23±0.52 (Buckfast x ARS-Y-C-1) to 6.68±0.73 mites observed in the ARS-Y-C-1 x Buckfast stock (P< 0.2568)(Appendix C). Significantly higher (P< 0.0001) numbers of mites were observed 14 days after the target bees were introduced into the host colony than after three days.

Discussion

ARS-Y-C-1 stock had been reported to display considerable resistance to A. woodi infestations under field conditions (Rinderer et al. 1993, Chapter IV of this dissertation). In this bioassay, similar strong levels of resistance were observed. ARS-Y-C-1 consistently maintained a low level of infestation of about 10% which was comparable to that of the Buckfast stock and the reciprocal F₁ hybrids. This observation corroborates the findings of Milne et al. (1991) and Lin et al. (1992) regarding the Buckfast stock.

Heavy infestations of A. woodi have caused tremendous damage to honey bees (Eischen 1987, Furgala et al. 1989, Otis & Scott-Dupree 1992). Tracheal mite infestations above 25% cause economic damage (Eischen et al. 1989, Otis & Scott-Dupree 1992). Our results indicate that by using ARS-Y-C-1 and the Buckfast stocks, the application of chemicals for tracheal mite control may be substantially reduced.
No increased resistance was observed in the reciprocal crosses. This suggests that both ARS-Y-C-1 and Buckfast stocks have similar mechanisms of resistance to tracheal mites, which are regulated by similar genetic characteristics.
References


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SUMMARY AND CONCLUSIONS

Controlling parasitic mites of honey bees using acaricides remains a serious problem worldwide because of hive products contamination, and possible development of acaricide resistant mite populations. The availability of honey bee stocks resistant or tolerant to mite pests will provide hope as a long term solution to these devastating parasites.

In this study, four selected stocks of honey bees (ARS-Y-C-1, Hastings, F₁ hybrid between ARS-Y-C-1 and Hastings stocks and Louisiana stock) were evaluated for resistance or tolerance to *Varroa jacobsoni* and to the three *Acarapis* species (*A. dorsalis*, *A. externus* and *A. woodi*) using choice (bioassay) and field experiments. Tracheal mite resistance of the ARS-Y-C-1 stock was also compared to that of Buckfast and their reciprocal hybrids using a bioassay.

Results showed a significant correlation between colony and queen mortality and levels of *Varroa* infestation in worker pupae. Considering dead colonies only, a tolerance index showed that Hastings, ARS-Y-C-1 and F₁ hybrid colonies exhibited significantly higher levels of tolerance to *V. jacobsoni*. These three stocks lived longer with higher levels of *Varroa* infestation in the brood cells. Louisiana stock was more susceptible to
Varroa infestation showing an earlier death with lower levels of infestation.

*V. jacobsoni* had similar reproductive success on the stocks tested based on the number of mites per infested pupa (1 to 10 mites), number of foundress *Varroa* per infested pupa (1 to 3 females), number of progeny per foundress (0 to 5 progeny), number of progeny per infested pupa (0 to 7 progeny), and proportion of infested pupae with infertile foundresses (0 to 47%). Apparently, differences in the duration of the capped stage of the four stocks did not influence the *Varroa*’s ability to reproduce. ARS-Y-C-1 stock had the longest capped period with the shortest duration observed in the F<sub>1</sub> hybrid colonies. Louisiana stock seemed to have a better grooming behavior, which may have regulated the population of *Varroa* to lower levels in this stock. Nonetheless, this characteristic did not influence the ability of Louisiana stock to tolerate mite infestations.

ARS-Y-C-1 and the F<sub>1</sub> hybrid colonies also showed considerable resistance to *A. woodi*. Colonies of these stocks consistently maintained about 10% tracheal mite infestations in two field trials. This level is well below 25%, the level reported to cause economic damage in honey bees. Resistance to *A. woodi* displayed by ARS-Y-C-1 stock was not significantly different from Buckfast stock and their reciprocal hybrids using a bioassay. Louisiana
and Hastings stocks had higher infestation levels of tracheal mites. *A. dorsalis* was most prevalent in the Hastings stock and levels of *A. externus* were higher on ARS-Y-C-1, F₁ hybrid and Louisiana stocks.

The mechanisms of resistance in the ARS-Y-C-1 and F₁ hybrid to *A. woodi* were not investigated. Therefore, further studies of this issue should be conducted.
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APPENDICES
Appendix A

Parameters on the Reproductive Success of
Varroa jacobsoni in Four Stocks of Apis mellifera
Evaluated for Resistance Using a Bioassay.
Appendix A.1. ANOVA and graph of the number of Varroa mites per infested pupa of four selected stocks of Apis mellifera. Unlabeled bars do not differ significantly at α=0.05 (LSMeans, SAS Institute, Inc. 1990).
Appendix A.2. ANOVA and graph of the number of foundress Varroa per infested pupa of four selected stocks of Apis mellifera. Unlabeled bars do not differ significantly at $\alpha=0.05$ (LSMeans, SAS Institute, Inc. 1990).
Appendix A.3. ANOVA and graph of the number of progeny per foundress Varroa of four selected stocks of *Apis mellifera* evaluated for resistance using a bioassay. Unlabeled bars do not differ significantly at $\alpha=0.05$ (LSMeans, SAS Institute, Inc. 1990).
Appendix A.4. ANOVA and graph of the number of progeny per infested pupa of four selected stocks of Apis mellifera evaluated for resistance using a bioassay. Unlabeled bars do not differ significantly at α=0.05 (LSMeans, SAS Institute, Inc. 1990).
Appendix A.5. ANOVA and graph of the proportion of infested pupae with infertile foundress Varroa. Unlabeled bars do not differ significantly at α=0.05 (LSMeans, SAS Institute, Inc. 1990).
Appendix B

Parameters on the Reproductive Success of *Varroa jacobsoni* in Four Stocks of *Apis mellifera* Evaluated for Resistance Under Field Conditions.
Appendix B.1. ANOVA and graph of the number of Varroa mites per infested pupa of four selected stocks of *Apis mellifera* for trial 1 (July 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
Appendix B.2. ANOVA and graph of the number of Varroa mites per infested pupa of four selected stocks of *Apis mellifera* for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
Appendix B.3. ANOVA and graph of the number of foundress Varroa per infested pupa of four selected stocks of Apis mellifera for trial 1 (July 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
No. of foundress/infested pupa

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No. of foundress/infested pupa over months 1990 to 1992.

- Hastings
- Louisiana
- $F_1$ Hybrid
- ARS-Y-C-1
Appendix B.4. ANOVA and graph of the number of foundress Varroa per infested pupa of four selected stocks of Apis mellifera for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly at $\alpha=0.05$ (SAS Institute, Inc. 1992).
Appendix B.5. ANOVA and graph of the number of progeny per foundress Varroa in four selected stocks of Apis mellifera for trial 1 (July 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
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<td>Stock x Month</td>
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### Graph

- **Y-axis:** No. of progeny/foundress
- **X-axis:** Years (1990-1992)
- **Legend:**
  - Hastings
  - Louisiana
  - F₁ Hybrid
  - ARS-Y-C-1
Appendix B.7. ANOVA and graph of the number of progeny per infested pupa of four selected stocks of *Apis mellifera* for trial 1 (July 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
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No. of progeny/infested pupa

- Hastings
- Louisiana
- F₁ Hybrid
- ARS-Y-C-1
Appendix B.8. ANOVA and graph of the number of progeny per infested pupa of four selected stocks of *Apis mellifera* for trial 2 (August 1991 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
Appendix B.9. ANOVA and graph of the proportion of infested pupae with infertile foundress Varroa in four selected stocks of Apis mellifera for trial 1 (July 1990 to June 1992). Unlabeled groups of stocks within months do not differ significantly (SAS Institute, Inc. 1992).
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% Infertile Foundresses

- Hastings
- Louisiana
- F₁ Hybrid
- ARS-Y-C-1
Appendix C

ANOVA and Graph of the Number of *Acarapis woodi* per Infested Bee of Five Stocks of Honey Bees. Unlabeled Bars Indicate Means are not Significantly Different (LSMeans, SAS Institute, Inc. 1990)

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<td>1446</td>
<td>237</td>
<td>0.0001</td>
</tr>
<tr>
<td>Time x Stock</td>
<td>4</td>
<td>6.55</td>
<td>1.07</td>
<td>0.37</td>
</tr>
</tbody>
</table>

![Graph showing the number of *Acarapis woodi* per infested bee for five stocks of honey bees. Unlabeled bars indicate means are not significantly different. The graph includes bars for Buckfast, ARS-Y-C-1, Buckfast x ARS-Y-C-1, ARS-Y-C-1 x Buckfast, and Louisiana stocks.]
VITA

Lilia Ibay de Guzman was born on September 11, 1959 to Juan and Aquilina Ibay in Urdaneta, Pangasinan, Philippines. She earned a Bachelor of Science degree in Agriculture in 1981 at Central Luzon State University. After graduation, she worked as a Research Assistant at the Research and Development Center's Apiculture project of the same institution.

In September 1986, she entered the Department of Entomology at Oregon State University. Her research concentrated on the biology of the two external species of *Acarapis* under the direction of Dr. D. Michael Burgett. She received her Master of Science degree in 1989.

She then joined the Department of Entomology at Louisiana State University for her doctoral degree in cooperation with the USDA/ARS, Honey-Bee Breeding, Genetics and Physiology Research. With the guidance and support of Dr. Thomas E. Rinderer, she studied the potential for tolerance or resistance of different stocks of honey bees to four species of parasitic mites.

She received the Hambleton student award in Apiculture from the Eastern Apiculture Society of North America, Inc. in 1993. Ms. de Guzman was married to Crisostomo de Guzman in 1983 and now has three children: Sheila, born in March 1989, and the twins Lorena and Marlou, born in August 1993.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Lilia Ibay de Guzman

Major Field: Entomology

Title of Dissertation: Tolerance Potential and Defense Mechanisms of Honey Bees (Apis mellifera L.) to Varroa jacobsoni Oud. (Acari: Varroidae) and Acarapis Species (Acari: Tarsonemidae)

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: April 7, 1994