BIOLOGY AND REARING OF *DIOMUS TERMINATUS* (COLEOPTERA: COCCINELLIDAE) ON *RHOPALOSIPHUM MAIDIS* (HOMOPTERA: APHIDIDAE) AND ARTIFICIAL DIET

By

KARIN MAJKEN HALLBORG

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OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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This research is dedicated to my parents Robert and Kathryn Hallborg
and to my boyfriend Casey Tifft.

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Karin Majken Hallborg

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Chair: Lance Osborne
Cochair: James Cuda
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A rearing system was developed for *Diomus terminatus* (Say), a coccinellid native to the Eastern and Midwestern United States, in order to evaluate these beetles' potential for the augmentative biological control of pest aphids. This system involved isolating three of the four life stages (egg, larvae, and adult) into separate containers in 5-day intervals, and providing either the aphid *Rhopalosiphum maidis* (Fitch) or *R. maidis* supplemented with artificial diet. Evaluation of larval survivorship, preoviposition period and sex ratio of the beetles indicated that these new methods improved quality and increased production when compared to previously described rearing methods The fecundity of laboratory-reared beetles using these two different feeding regimes (aphids only and supplemented with artificial diet) also was evaluated. There was no significant difference in fecundity due to larval nutrition for adults that consumed 4-6 aphids per day (7.71 ± 5.61 and 8.23 ± 5.53). However, at higher levels of aphid consumption (7-10

aphids per day) those beetles exposed to artificial diet as larvae laid more eggs(10.95 ± 5.92 eggs per day) compared to those not exposed to artificial diet as larvae (14.13 ± 8.14 eggs per day). The results of median eggs fertility were lowest (69.2%) when adults were provided only one aphid per day, but increased significantly to 80.0% when the single aphid was supplemented with artificial diet.

CHAPTER 1
REVIEW OF LITERATURE

This review of the literature will describe the significance of horticulture to the Florida economy, as well as the most common insect pests, and current pest insect management strategies in Florida. The trends in biological control towards using native beneficial insects, and the use of artificial diets in mass production also will be reviewed. Finally, the general biology of aphids and lady beetles, and a detailed description of the known biology of the native predator *Diomus terminatus* (Say) (Coleoptera: Coccinellidae) will be provided.

Horticulture in Florida

Florida ranks fourth in the United States in total crop production with cash receipts of $5.354 billion in 1998 (Florida Agricultural Statistics Service, 2003). Currently, Florida is ranked first nationally in citrus, sugar, and tomato sales, and second in ornamental plant and vegetable sales. The leading crops in Florida are vegetables, melons and berries with $1.7 billion in cash receipts, and 25.48% of total agricultural sales. Citrus products had cash receipts of $1.6 billion and were 24.04% of total agricultural sales in 1998. Nursery crops had sales of $1.3 billion, and 19.99%, and field crops had cash receipts of $645 million or 9.65% of Florida agricultural sales (Florida Agricultural Statistics Service, 2003).

Integrated Pest Management

The costs of insect pest management can be a significant part of a grower's budget. A 1995 survey of 221 Florida ornamental growers found 20% spent 16-20% of their

production budget on pest management, including materials, labor, and equipment (Hodges et al., 1998). According to Hodges et al. (1998), almost all respondents (95%) reported using insecticides and miticides, and 15% reported using biocontrols. The most common arthropod pests were mites, aphids, whiteflies, scales, thrips, leafminers, borers and caterpillars. Growers listed 32 insecticides among their pest control arsenal, with many growers using more than one insecticide. The most common insecticides used were acephate (59%), diazinon (43%) and avermectin (43%) (Hodges et al., 1998). The cost of pest management is often significantly more expensive in ornamental crops due to the nature of the industry. While actual plant damage is often less than 15%, the cosmetic damage to the leaves and stem can make an ornamental plant unmarketable (Osborne et al., 1994).

Alternatives to chemical control are in demand because of the negative side effects from continued use of pesticides such as phytotoxicity, secondary pest outbreaks, and pesticide resistance. Additionally, the public is demanding more organic and pesticide-reduced crops (Hale and Elliott, 2003). Insecticide resistance has been accelerated in important pests such as aphids, mites, and whiteflies due to their ability to avoid chemical pesticides by residing on the underside of leaves. This leads to multiple pesticide applications and an increase in resistance to the pesticide (Hussey and Scopes, 1977). Moreover, resistance to one material may lead to cross-resistance to all other pesticides in that family with similar modes of action (Hussey and Scopes, 1977).

Integrated Pest Management (IPM) incorporates multiple pest management practices including cultural, biological, physical, as well as chemical treatments, with the objective of reducing pesticide use while maintaining pests below economic injury levels.

The trend towards IPM arose from concerns of pesticide residues in the ecosystem, as well as public pressure. In 1993, the United States Depatement of Agriculture, Environmental Protection Agency and the Food and Drug Administration announced a the goal of implementing IPM on 75% of U.S. cropland (USDA/CSREES, 1996). In addition, the Food Quality Protection Act passed by the U.S. Congress in 1996 mandated that the safety of pesticides be demonstrated prior to being labeled for commercial use (U.S. Congress, 1996). The high cost of these tests, which are paid for by the chemical manufacturing industries, has resulted in the lack of pesticide registration on many minor crops, such as ornamentals, as well as the removal of registration on other crops (Capinera et al., 1994). While the volunatary or involunatary reductions in pesticide availablity are regretted by some, many researchers believe the use of nonselective insecticides may actually aggravate pest control problems by killing predators and parasitoids that aid in the control of pest insects (Obrycki and Kring, 1998).

Biological Control

One facet of IPM is biological control. Biological control is the suppression of pest populations by natural enemies (US Congress Office of Technology Assessment, 1995). Biological control typically is accomplished through the use of beneficial insects or other arthropods which prey upon, or parasitize pests, or through microbial pathogens which cause disease in pests (Capinera et al., 1994). The National Academy of Sciences, an organization mandated by the U.S. Congress to advise the federal government on scientific and technical matters, stated that "biological control can and should become the primary method used in the United States to ensure the health and productivity of important plant and animal species" (National Academy of Sciences, 1988, pp.68).

Biological control takes many forms. Classical biological control is the release of natural enemies from the native range of the invasive pest with the goal of permanent establishment (Flint and Gouveia, 2001). For example, the vedalia beetle *Rodolia cardinalis*, Mulsant (Coleoptera: Coccinellidae), was introduced from Australia and is credited for rescuing the California citrus industry from collapse due to damage from the Cottony Cushion Scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae) (Weeden et al., 2003). Augmentative biological control is the repetitive release of laboratory-reared or field collected beneficial organisms (Flint and Gouveia, 2001).

Not all predators and parasitoids are suitable for use in a biological control program. Doutt and Debach (1964) compiled a list of important characteristics for biological control agents. Accordingly, a successful natural enemy should exhibit the following characteristics: 1. High searching capacity, or ability to find the host at low densities. 2. Be fairly host specific and have a direct dependence on changes in the host's population. 3. A potential rate of increase that is very high, due to a short development time and relatively high fecundity. 4. Survive well in all of the niches occupied by the host. Additionally, although not a requirement, ideally the biological control agent can be mass-produced.

Increasingly there have been concerns raised regarding introduced biological control agents on native organisms, and the non-target effects of these potentially permanent non-native species (Simberloff, 1992; Simberloff and Stiling, 1996; Strong and Pemberton, 2000; Thomas and Willis, 1998; Michaud, 2002). Some insects released as biological control agents have been documented to attack non-target native organisms (Strong, 1997) and escape into native habitats (Louda et al., 1997; Ryan, 1997). The non-

target effects from these insects include direct predation, herbivory, and parasitism, as well as competition with native, non-target species. These interactions may cause unexpected community and ecosystem damage (Simberloff and Stiling, 1996). The introduced lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) is now considered an "invasive biological control agent" because it displaces native coccinellids, consumes non-target, non-pest organisms, and becomes a nuisance when large aggregations attempt to overwinter in homes (Michaud, 2002, pp. 534). While the effects of releasing non-native natural enemies are often hard to determine due to inadequate monitoring, the lack of clear examples of non-target effects does not mean the effects do not occur (Simberloff and Stiling, 1996).

Native beneficial insects often are overlooked as viable biological control agents. However, it is unlikely that the invaded ecosystem is without any natural enemies capable of preying upon the non-indigenous organism (Michaud, 2002). Given time, an invasive species may be brought under control by generalist predators already present, as was the case with the brown citrus aphid, *Toxoptera citricida* (Kirklady) (Michaud, 2002), and for the citrus leafminer, *Phyllocnistis citrella* Stainton in Florida (Amalin et al., 2001; Amalin et al. 2002).

Diomus terminatus is a coccinellid native to the Eastern and Midwestern United States. The home range encompasses Texas to Vermont including the entire state of Florida (Gordon, 1976), and has been documented as far east as Bermuda (Hilburn and Gordon, 1989). It survives in a wide range of habitats and environmental conditions, and feeds on a variety of aphids found in these areas. Mass-producing and releasing *D. terminatus* for the augmentative biological control of pest aphids found in Florida should

be considered. There are far fewer risks associated with the release of native organisms than introduced species, and it is already evident that the native natural enemy can survive in the local environment.

Insect Mass Rearing and Artificial Diets

The high cost and unpredictability in supply and quality of beneficial insects is a major reason why many growers use pesticides over biological control for pest management (Penn et al., 1998). Efficient mass rearing systems that produce large numbers of high quality natural enemies are the pre-requisite to the widespread adoption of a biological control strategy. In order to develop a beneficial insect rearing program, a basic understanding of the insect's biology is essential (Waage et al., 1985). Different entomophagous insects have different life histories, behaviors and nutritional requirements. The initial investigation should involve observing the insect in nature. Knowledge of insect behavior in the field will help determine if the insects are behaving normally in the laboratory (Brooks, 1984). A thorough study of the existing literature on the biology and rearing methods of related insects will provide the investigator a starting point, and may provide insight into future problems and potential solutions.

When the colony is initiated the insects should be provided with as similar an environment as possible in containment as is found in nature. From this point, progress can be made in refining and improving the efficiency of the rearing program. In this project, efficiency was improved by answering the following questions: Where have the insects been documented or observed to exhibit performance in terms of reproduction and development? Are the proper substrates provided? Can reproduction and development occur on alternative substrates? Are the proper stimuli present, such as volatile compounds from the plant or host? Once these initial questions are understood and

incorporated into a laboratory rearing system, modifications for the transition to mass-production can be investigated. These include the number of insects per container to optimize egg production and larval survivorship, efficient harvesting techniques, and alternative foods that may improve fecundity and fertility.

The history of insect rearing on artificial diets spans close to 100 years beginning with the Bogdanow diet (1908) for *Calliphora vomitoria* (L.), which consisted of peptone, meat extract, starch and minerals (Bogdanow, 1908 [as cited in] Singh and Moore, 1985). Artificial diets that have been used to rear multiple generations of insects have been compiled in books by House (1967), House et al. (1971), and Singh and Moore (1985).

The development of an effective artificial diet is a very complicated process involving a thorough understanding of insect behavior, biology and physiology. Many predators will only eat living, moving prey. Some predators use complex chemical cues to elicit prey searching behavior and acceptance. These chemicals must be identified, manufactured and incorporated into the diet. Even with proper physical and chemical stimuli, the nutrients in the diet must be qualitatively and quantitatively appropriate to support the normal growth, development, fecundity and behavior of the insect (Cohen, 1992).

Insect mass production borrows from many disciplines. In tritrophic rearing programs involving growing the prey's host plant, an understanding of plant propagation, physiology, pathology, and nutrition is necessary. This is in addition to an understanding of other areas of entomology such as physiology, morphology, biometry, nutrition, pathology and behavior needed to develop an efficient insect-rearing program (Brooks,

1984). Designing the proper facility involves an understanding of environmental controls, and may incorporate greenhouse design, lighting and irrigation.

Aphid Pest Management

Aphids (Homoptera: Aphididae) cause damage by sucking nutrients from plants, vectoring viruses, causing deformation at the growing tips, and by producing honeydew (Fransen, 1993). Honeydew, the sugar-rich excrement of many Homoptera, cause additional damage to the plant by clogging stomata and encouraging the growth of dark-colored sooty mold (*Capnodium spp, Fumago spp.* and other fungi) on the leaves and stems, which can significantly reduce photosynthesis (Rabassee and Wyatt, 1985).

Aphids are difficult to control due to their extremely high fecundity. Aphids reproduce parthenogenetically, reproducing females without fertilization. A female aphid produces 50-100 daughters in her life span, with each daughter reproducing in 6-8 days (Short, 1993). Aphids can reproduce three to six times a day for several weeks depending on environmental conditions, the species of the aphid and host plant, and plant vigor (Rabasse and Wyatt, 1985).

Currently, the most common treatments for aphids on ornamental plants are chemical insecticides such as acephate, diazinon, and malathion (Hodges et al., 1998). However, the economically important aphids common in Florida, *Aphis gossypii* Glover and *Myzus persicae* (Sulzer), have demonstrated resistance to several classes of insecticides (Anthon, 1955; Furk et al., 1980; McClanahan and Founk, 1983; Furk and Hines, 1993). The biological control of pest aphids has been attempted with commercially available natural enemies with varying degrees of success. These included green lacewings, *Chrysoperla spp*., (Neuroptera: Chrysopidae), the midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), and the parasitoids *Aphidius colemani*

Viereck, and *Aphidius ervi* Haliday (Hymenoptera: Braconidae), the lady beetle *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), and the entomopathogenic fungus *Verticillium lecanii* (Zimmerman) Viegas (Hypocreales: Hypocreacae). However, in the high heat and humidity conditions common in Florida, these biological control agents have not been able to keep the aphid populations below economic thresholds (Osborne et al., 1994)

In a trial conducted by Parrella and Heinz (1998), sufficient aphid biological control was achieved with parasitic wasps in greenhouses located in the cooler climates of Europe (45-53° N.). However, in warmer climates farther south (30-45° N.), and during the summer when greenhouses are more open, and aphid suppression was less pronounced. This was due in part because the interaction between parasitoids and aphids is not stable. Parasitic wasps of aphids tend to have long development periods followed a short fecundity period (Wyatt, 1985). This condition is a violation of Doutt and Debach's (1964) third criterion for suitable biological control organisms. Aphids reproduce and develop more quickly than wasps causing the aphid/aphid-parasitoid generations to become unsynchronized. This leads to a lull of activity in which aphids continue to feed and reproduce unchecked (Wyatt, 1985). While coccinellids also have a longer developmental period than aphids, this is offset because coccinellids are voracious feeders even as larvae.

While host-specificity is an important criterion according to Doutt and Debach (1964), it can also limit the success of a biological control agent in periods of food shortage. Aphid parasitoids are rather species-specific and typically do not parasitize all the aphid species found in a growing environment. Hence, the grower must be able to

positively identify the aphid species infesting the crop in order to introduce the appropriate parasitoid (Van Steenis, 1993). In addition, the aphid must be at the appropriate age or size for parasitization. However, generalist predators such as coccinellids are able to feed, both as larvae and adults, on aphids regardless of the age or development of the prey.

Predaceous coccinellids are used in biological control more often than any other taxon of predatory organisms, and have a proven success record in biological control that is known even to the general public (Obrycki and Kring, 1998). Coccinellids have a high searching capacity, occupy various habitats, and tend to exploit all niches of their prey. They have a wide range of acceptable food (polyphagy) including pollen, and also are cannibalistic, which allow them to survive in periods of food shortage. When prey is abundant they are voracious feeders both as larvae and adults, and demonstrate a strong tendency towards increased oviposition with increased food consumption (Hodek and Honek, 1996). These characteristics satisfy several of the criteria listed by Doutt and Debach (1964).

***Diomus terminatus* (Say)**

The native coccinellid *D. terminatus* has been suggested for the biological control of greenhouse aphids (Osborne, 2000) and the sugarcane aphid, *Melanaphis sacchari* (Zehnter) (White et al., 2001). Little has been published in the scientific literature regarding the rearing of *D. terminatus*. Hall (2001) determined that *D. terminatus* laid an average of 3.0 eggs per day for 17.0 ± 7.9 days, for an average total of 41.9 eggs ± 54.8 (n=22) when fed the yellow sugar cane aphid, *Sipha flava* Forbes. However, prior unpublished experiments on *D. terminatus* conducted in the laboratory of L.S. Osborne (unpublished) showed a much more positive picture. The results from these trials

demonstrated a higher number of eggs laid (85.5 and 37.1) during the lifespan of the beetle when fed *A. gossypii* (n=26) and *M. persicae* (n=25) respectfully. Aphid consumption averaged between 5-13 aphids per day for *S. flava, M. persicae* and *A. gossypii* (Hall, 2001; Hentz and Nuessly, 2002; Osborne, unpublished).

Research Goals and Justification

This research is part of a larger project to evaluate the efficacy of this native predator for the augmentative biological control of pest aphids in Florida. The experiments conducted were designed to elucidate the biology of *D. terminatus* including aphid consumption and fecundity, preoviposition period, egg fertility, egg to adult survivorship, sexual dimorphism and sex ratio. In order to mass-produce this beetle, an efficient rearing method for *D. terminatus* on aphids alone, as well as a diet of aphids supplemented with an artificial diet was developed and evaluated.

CHAPTER 2
REARING *DIOMUS TERMINATUS* (COLEOPTRA: COCCINELLIDAE) FOR THE AUGMENTATIVE BIOLOGICAL CONTROL OF PEST APHIDS (HOMOPTERA: APHIDIDAE)

Introduction

Diomus terminatus (Say) is a coccinellid indigenous to the Eastern and Midwestern United States (Gordon, 1976) and demonstrates potential as a biological control agent of pest aphids (White et al., 2001; Osborne, 2000). Because *D. terminatus* is also native to the Southeast, it is adapted to the habitat, environmental conditions, and prey found in Florida. In contrast, there are concerns regarding the risks of non-target effects of potentially permanent, introduced organisms on native species (Simberloff, 1992; Simberloff and Stiling, 1996; Strong and Pemberton, 2000; Thomas and Willis, 1998). Michaud (2002) suggests that in many cases, native natural enemies can provide sufficient biological control, and that research in biological control should focus on native beneficial organisms.

Little has been published in the scientific literature regarding the rearing of *D. terminatus*. Hall (2001) determined that *D. terminatus* laid an average of 3.0 eggs per day for 17.0 ± 7.9 days, for an average total of 41.9 ± 54.8 eggs (n=22) when fed the yellow sugar cane aphid, *Sipha flava* Forbes. However, prior experiments on *D. terminatus* conducted in the laboratory (L.S. Osborne, unpublished) showed a much more positive picture. The results from these trials demonstrated a higher number of eggs laid, 85.5, during the lifespan of the beetle, when fed *Aphis gossypii* (Glover) (n=26) and 37.1 on a diet of *Myzus persicae* (Sulzer) (n=25). Although the average life span was not

determined, one *D. terminatus* had a life span of 143 days when fed *A. gossypii* and another survived 75 days on *M. persicae.* Hentz and Nuessly (2002) reported several beetles survived for 50 days entirely on a diet of *S. flava*, although the average life span was not recorded.

No data were collected on adult feeding rates, but Hall (2001) observed that adults seemed to consume 5-10 aphids per day. In another laboratory study, average daily consumption rates of 13.5 *A. gossypii* (n=36) and 8.7 *M. persicae* (n=25) were observed (L.S. Osborne, unpublished). The total number of aphids consumed during the lifespan of *D. terminatus* averaged 425.5 (n=36) for *A. gossypii* and 243.3 (n=25) for *M. persicae*.

Hall (2001) and Hentz and Nuessly (2002) reported similar developmental times for embryogenesis (3-4 days) and pupation (4-5 days) when fed *S. flava*. However, there were substantial discrepancies in the length of time spent in the larval stage. Hall (2001) reported the larval stage lasted 10 days, while Hentz and Nuessly (2002) reported 4 days. The development of the egg, larvae, and pupae stadia were 6.3, 9.4, and 6.4 days, respectively, when fed a diet of *A. gossypii*, and 6.2, 7.4, and 4.1 days respectively when provided *M. persicae* (n=28) (L.S. Osborne, unpublished).

The wide range in the number of eggs laid, larval developmental period, and adult longevity, indicate there is room for improvement in the rearing of *D. terminatus*. The longer life span and higher fecundity of *D. terminatus* when provided *A. gossypii* and *M. persicae* suggest that while this predator may not be ideally suited to *S. flava*, it may be valuable in the biological control of other economically important aphids.

In order to better assess the potential of *D. terminatus* as a biological control agent against pest aphids, including its suitability for mass production, a novel rearing system

was developed. This system is described in this chapter. In order to evaluate this rearing method in comparison to previously described methods, trials were conducted to elucidate egg to adult survivorship, preoviposition period, and sex ratio using the novel rearing method.

Materials and Methods

Sorghum Production

Sorghum (*Sorghum bicolor* var. hybrid grain sorghum SS800) was grown in a greenhouse (23° C ± 6° C, 70% RH, ambient light) until it was ready for introduction into the aphid colony, approximately 4 weeks after planting. To produce the plants, two seeds were placed in a 6-cm x 5.5-cm compartment of a seedling tray containing a soil mixture of 2 parts peat: 1 part perlite: 1 part vermiculate. During the winter months when there was insufficient heating in the greenhouse, it was necessary to germinate the seeds in the laboratory using germination paper in tightly sealed containers. In warmer months, the sorghum germinated sufficiently in the greenhouse. A slow release fertilizer (Dynamite® 6 month 18-6-8 (N: P: K) plus micronutrients, Enviro-Safe Laboratories, Miami, FL) also was added to the soil during the winter months. After germination, the seedlings were thinned to one plant per compartment. Plants were fertilized with Peters® 20-20-20 (N: P: K) (United Industries Corp., St. Louis, MO) at the label rate (1 cc: 1 L) two times per week and watered as needed in between fertilization. Sorghum grew to sufficient height in the seedling container and did not need to be transplanted into larger containers prior to introduction to the aphid colony. Plants were ready for introduction into the aphid colony approximately 4-6 weeks after seeding, when they were at least 40 cm tall.

Aphid Colony

The *R. maidis* colony was maintained in three Florida Reach-In® growth chambers (Walker et al., 1993) at the University of Florida Entomology and Nematology Department, programmed for 22° C with a decreasing photoperiod of 16 hours full light (1399 Lux ± 10%), 2 hours reduced light (334 Lux ± 10%) and 6 hours darkness. There were two 20-watt fluorescent bulbs in the back of the growth chamber behind Plexiglas®, with two 20-watt white fluorescent bulbs (Sylvania® Cool White), and two 20-watt blue/white bulbs fluorescent (Sylvania® Gro-Lux Aquarium) overhead. Humidity was approximately 50%.

In total, 48 plants were placed in a solid-bottom tray in the growth chamber. The new plants were inoculated with aphids from another aphid colony by inserting aphid-infested leaves directly into the whorl of the plant. This succulent part of the plant encourages rapid aphid reproduction. The new colony was ready for use in 1 week.

In order to insure no accidental introduction of aphid predators or parasitoids, each chamber was cleaned with Windex® and remained empty and dark in between introductions of fresh plants. All plants were thoroughly examined, and pests removed, prior to introduction to the colony, and three times a week thereafter. The best method to insure that the aphid colony was kept free from natural enemies was by keeping the colony indoors, and far from the *D. terminatus* colony. Contamination by entomopathogenic fungi was avoided by maintaining a low humidity, and by directing water toward the roots of the plants, and carefully avoiding splashing the leaves or stems. Each individual aphid colony was used within 3 weeks to insure it did not "crash" due to plant stress, predators, parasitoids, or fungi.

Containerization

Diomus terminatus were raised in containers (Pioneer Packaging, Dixon, KY), rather than on the whole plant in order to conform to space limitations and to facilitate harvesting. The *D. terminatus* colony was maintained in round containers 21-cm in diameter by 7.5 cm high, with two 4-cm diameter, or three 3-cm diameter ventilation holes drilled into the lid (Figure 2-1). Humidity within the containers was maintained at 70-90% using tape to open or close the ventilation holes. Low humidity could be raised by misting the top of the container with water, or by providing a moist cotton ball. Each container had a rack made of 1-cm mesh hardware cloth folded so that it stood approximately 4-cm from the bottom of the container. Two rolled laboratory tissues (Kimwipes® Ex-L, 38.1 x 42.6 cm, Kimberly-Clark, Roswell, GA) were placed under the rack to absorb excess moisture and to provide pupation sites. Several wax paper strips (22-cm x 2-cm wide) were provided for hiding and egg deposition.

Figure 2-1. Rearing container for *D. terminatus* showing the three 3-cm taped ventilation holes.

Five-day Rotation

There were two key factors that were relied upon in the development of the five-day container rotation system that has been successfully used for 25 weeks. (1) It required approximately 3 to 4 days for the eggs of *D. terminatus* to hatch, 5 to 10 days to enter the pupal stage and 3 to 5 days to emerge as an adult at 27° C, 16L: 8D (Hall, 2001; Hentz and Nuessly, 2002; L.S. Osborne, unpublished). (2) Cannibalism was a significant mortality factor in rearing *D. terminatus*. Cannibalism was reduced by providing sufficient amounts of favorable food as well as by grouping the individuals by stage of development. This rotation system also reduced the possibility of accidentally discarding the elusive eggs, and made harvesting pupae easy.

Thirty adult beetles were placed in a container in the growth chamber for 5 days. The beetles were fed by placing aphid-infested sorghum leaves directly on the hardware cloth rack at the rate of approximately 10 aphids per beetle. The beetles laid eggs (0.68 ± 0.001 mm long by 0.37 ± 0.001 mm wide) singly, mainly on the leaves, but also on the wax paper and Kimwipes® during the five-day period. After 5 days, the adults were removed from the container and the remaining leaves, Kimwipes®, and wax paper became the "nursery." Larvae were fed at the rate of approximately five aphids per larvae in the first and second instar, and seven aphid per larvae in the third and fourth instar. By day 10, the majority of the larvae had pupated and there were only a few newly eclosed adults (Figure 2-2). Because the majority of the pupae were on the Kimwipes®, harvesting pupae was usually simply a matter of collecting the Kimwipes®.

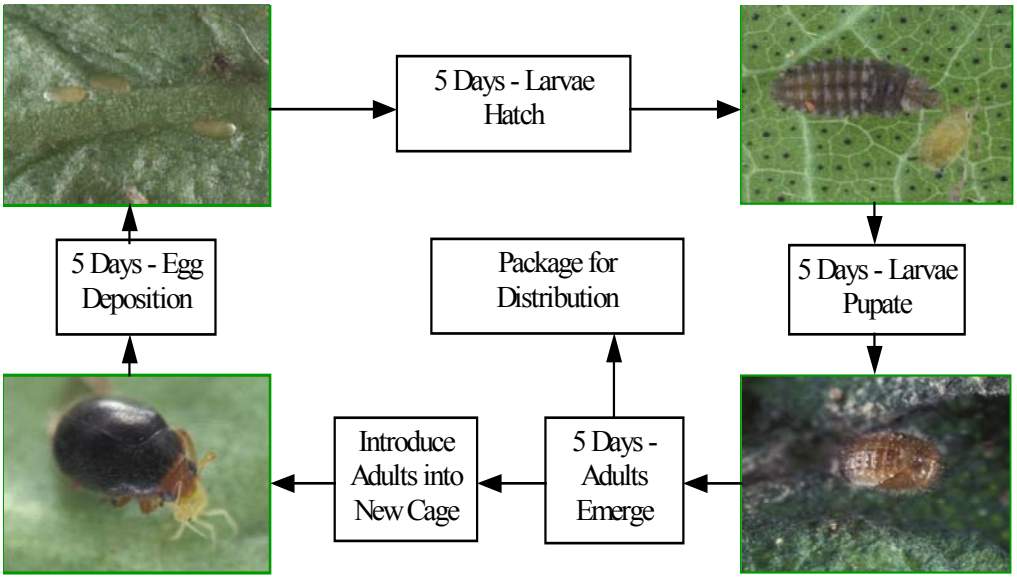

Figure 2-2. Schematic diagram of the 5-day rearing system for *D. terminatus*

Harvesting

The most efficient method for harvesting *D. terminatus*, using the five-day containerized rearing system, was to collect the pupae and aspirate the adults upon emergence. Most pupae were found on the Kimwipes®, with a very few on the desiccated leaves or on the lid of the rearing container. Adults were harvested by aspiration or by phototaxis. In the first method, the tissue paper collected from the bottom of the rearing container was carefully opened in a light box and the beetles aspirated. This technique was easiest to perform with rolled, not crumpled, Kimwipes®.

In the second method, a large white industrial funnel (Gemplers, Bellville, WI) was inverted and replaced the lid of the container. The funnel must fit tightly to avoid beetle escapes. A small container (10-cm x 7.5-cm) containing bee pollen, free water or a moist cotton ball, and wax paper strips (10-cm x 2-cm) for hiding, was placed on top of the funnel. Two lights were used, one focused directly on the funnel and the other directed on the top of the container. The beetles crawled into the funnel, toward the light, and become trapped in the container (Figure 2-3).

One problem with the light harvesting method was that while it was less labor intensive, it took longer to collect the insects, and not all the beetles were collected. Adults typically emerged by the third or fourth day after collection as pupae, but it required up to 10 days for most of the beetles to find their way to the collection container at the top of the funnel. Even then, not all the beetles were collected. Therefore, this technique is only recommended in a high production system where a long harvesting period and an incomplete harvest is acceptable.

To determine if these novel rearing methods were suitable improvements over the methods described by Hall (2001) and Hentz and Nuessly (2002), measurements were taken of the following parameters: egg to adult survivorship, preoviposition period, and sex ratios.

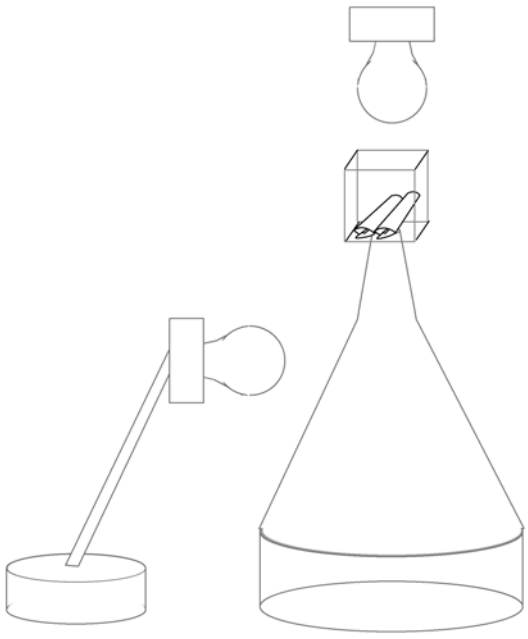

Figure 2-3. Diagram of light harvesting technique. A funnel is inverted on top of the container with a capture container on top of the funnel. One light shines directly on the funnel, and the other light shines on the top of the container.

Egg to Adult Survivorship

To determine the percentage of adults that survived from a single clutch of eggs, 20 clutches of eggs were collected over 3 days from seven individuals. To collect the eggs, females were isolated in small, lipped Petri dishes (5 cm x 0.9 cm) and provided at least 20 aphids, a strip of wax paper (4 cm x 1 cm) and a droplet of water. After 24-hours the beetle was removed and the number of eggs counted. An abundant amount of aphids (> 7 aphids/larvae) and a droplet of water were provided daily to the larvae. The number of adults that eclosed 10 days later was counted. The average percent survival was then calculated.

Preoviposition Period

It is important to know the preoviposition period of an insect when investigating rearing methods in order to allow ample time for mating. The length of time between eclosion and when the first eggs are laid also can vary depending on the quality or health of an insect (N.C. Leppla, personal communication). The purpose of this trial was to determine the preovipositon period of *D. terminatus* when fed *R. maidis* nymphs.

Diomus terminatus pupae were collected on the same day from all colony cages. Virgin adults were collected within 24-hours of eclosion and five adults were placed in each of 12 containers measuring 10-cm wide by 10-cm long and 7.5-cm high. Each container was supplied with 10 aphids per beetle per day, water, and two strips of wax paper (10 cm x 2 cm), and sealed with Parafilm® to ensure neither the beetles nor the aphids escaped. The beetles in three containers were presented aphids on a sorghum leaf. The wax paper, leaves, and container were thoroughly examined daily for eggs, and fresh aphids were provided. This procedure continued until eggs were observed in all the cages.

Sex Ratios

The successful mass rearing of an insect also requires knowledge of the average sex ratio of a cohort to insure a proper balance between females and males for reproduction. As is common in many coccinellids, there are few external differences between male and female *D. terminatus*; therefore, adults were dissected to determine gender. Three cohorts, totaling 144 pupae, were collected from the colony. Adults were frozen at -10° C upon emergence. Dissections were made under an 80x-dissecting microscope in 70% ethanol. The gender of the beetle was confirmed when the male aedeagus (Figure 2-4) or female spermatheca (Figure 2-5) was identified. Data were analyzed by binomial proportions software available on Minitab(Minitab Statistical Software, 2000).

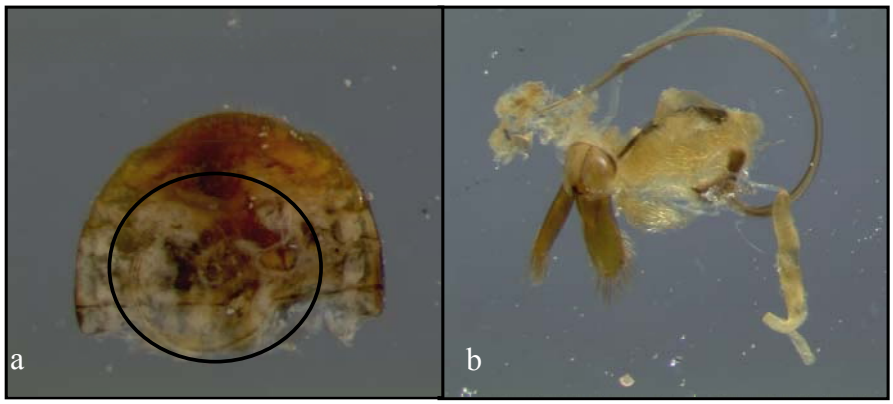

Figure 2-4. Male *D. terminatus* (a) Ventral view of abdomen. The aedeagus (circled) identifies this individual as male. (b) Excised aedeagus.

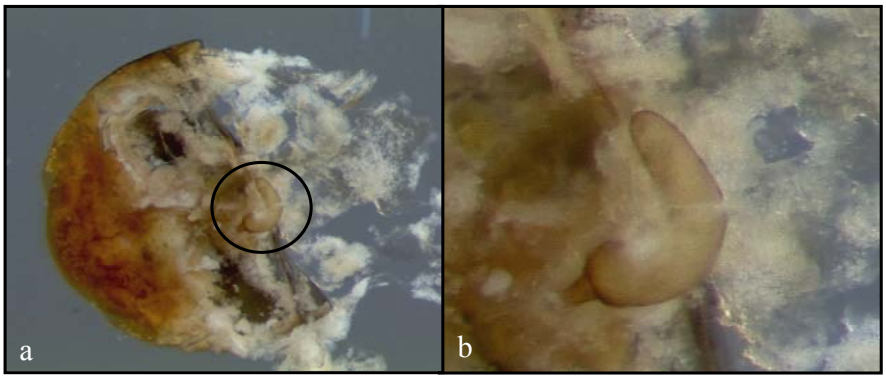

Figure 2-5. Female *D. terminatus* (a) Ventral view of abdomen. The spermatheca (circled) indicates this is a female. (b) Enlarged image of spermatheca.

Results

Five-Day Rotation

Using the methods described above, we typically collected 20-40 pupae or freshly emerged adults every 5 days. Collections were greater when excess food was provided. This system was used for 25 weeks, and for over 10 generations.

Egg to Adult Survivorship

The average percent of *D. terminatus* that completed egg to adult development was 38.8% ± 0.05 SE . This average is based on a wide range (7-93%), which may be due to higher cannibalism rates when more eggs were laid, or an insufficient supply of aphids.

Preoviposition Period

Eggs were seen in all containers within 7 days after adult eclosion. Beetles in containers with sorghum leaves laid eggs within 4 days. Of the eight containers without leaves, three had eggs present on the fourth day, three more containers had eggs on day 5, and one more container each had eggs present on days 6 and 7.

Sex Ratio

In total, there were 70 females and 74 males produced from the three cohorts (Table 2-1). Overall, there were no significant differences in the sex ratios between the cohorts (P=0.803). In addition, the sex ratio within each cohort was not skewed (Cohort 1: P=0.302, Cohort 2: P=1.000, Cohort 3: P=0.551).

Table 2-1. Sex ratios for three cohorts of *D. terminatus*

Gender	Cohort 1	Cohort 2	Cohort 3	Total
Female	33	12	25	70
Male	43	11	20	74
Ratio (F: M)	0.77: 1.00	1.00: 1.09	1.00: 1.25	1.00: 0.95

Discussion and Conclusions

The rearing methods detailed in this paper described several significant changes from the methods reported by Hall (2001) and Hentz and Nuessly (2002). These differences included feeding *D. terminatus* the aphid *R. maidis* rather than *S. flava,* using larger rearing containers and larger Petri dishes, and presenting the aphids on sorghum leaves. Due to difficulties in rearing *S. flava* in the summer and fall, Hall (2001) was unable to mass-produce *D. terminatus.* We had similar problems in the summer, but were able to rear *R. maidis* indoors in growth chambers. In addition, Hall (2001) used younger sorghum plants, which tend to exhibit physiological stress faster due to heat and pest pressure than more mature plants. By incorporating these modifications, our results showed that the mass production of high quality *D. terminatus* was feasible.

According to the methods of Hall (2001), the beetles reared in large glass tubes (15-cm x 2.2-cm) produced an average of 1.4 ± 1.5 eggs per day. However, females isolated in glass microtubes (7.4-cm x 0.9-cm) laid twice as many, or 3.0 eggs/day. In preliminary trials we found *D. terminatus* produced 14.13 ± 8.14 eggs per day when provided 7-10 aphids per day. There are two hypotheses for the discrepancy in fecundity. *Rhopalosiphum maidis* may be nutritionally superior to *S. flava*, and may therefore lead to an increased fecundity. The second hypothesis is that the different petri dishes used in our study may be more suitable for egg deposition due to the size and texture of the arena.

Hall (2001) also used a brushing machine to remove the aphids from sorghum leaves before feeding them to *D. termintus*. We found this step unnecessary, and it may even be detrimental by denying the beetle the chemical cues found in plant volatiles, which may stimulate or increase egg deposition. Additionally, the aphids may be killed

or damaged by the brushing machine, which may make the aphids less appealing and thereby reduce aphid consumption and subsequent beetle fecundity. Evidence for this was demonstrated in the slightly shorter preoviposition period that occurred with beetles provided aphids on sorghum leaves compared to those where the aphids were removed from the leaves.

Although fecundity increased by using the methodology developed for this study, the differences in rearing methods did not increase the average percentage of eggs that developed to the adult stage. The average survivorship on *S. flava* was 39.4% (Hall, 2001) and 38.8 % ± 0.05 % in our system. Survivorship may be improved by providing adequate shelter and hiding spaces, and by providing an abundance of food, as cannibalism is a major mortality factor for eggs, larvae and pupae.

Hentz and Nuessly (2002) found the preoviposition period of *D. terminatus* to be about 7 to 8 days when fed *S. flava*. It is not possible to positively state that *D. terminatus* has a shorter preoviposition period when fed *R. maidis* than *S. flava* because their methods were not described in detail. However, other researchers have demonstrated that differences in the preoviposition period can be attributed to the aphid species consumed, and the amount of prey provided (Hodek and Honēk, 1996). *Harmonia axyridis* Pallas reared on *M. persicae* had a preoviposition period that was 2.2 days shorter than when fed *Amphorophora oleracea* (van der Goot), now a junior synonym of *Hyperomyzus carduellinus* (Theobald) (Hodek and Honēk, 1996). The coccinellid *Propylea japonica* (Thunberg) had a preoviposition period of 9.6 days, an average clutch size of 6.7 ± 3.3 eggs, and laid only 109 eggs when it consumed 19 *A.gossypii* per day. However, there was a significant decrease in the preoviposition

period (to 4.3 days), and an increase in average clutch size (9.1± 3.9), and total lifetime fecundity (1481 eggs) when 73 *A.gossypii* were eaten per day (Hodek and Honēk, 1996). *Diomus terminatus* also seemed to have an increased adult longevity when fed *R. maidis* as compared to *S. flava*. Although no quantitative measurements were taken, *D. terminatus* consistently survived 30 days in the rearing containers, while Hall (2001) reported an average lifespan of 17.0 ± 7.9 when fed *S. flava*. Based on the life history parameters measured (sex ratio, egg to adult survivorship and preoviposition period) it is evident these novel methods for rearing *D. terminatus* have improved both the mass-rearing potential and quality of this beetle compared to those evaluated by Hall (2001) and Hentz and Nuessly (2002). While we have made refinements in the rearing techniques over the last two years, the authors fully expect further refinement. The methods described in this paper were only performed on a laboratory scale, and have yet to be converted to a mass-production scale. Further experiments must be performed to determine the optimum number of beetles per cage, and the optimum prey food for maximum fecundity and fertility. A long-term goal is to find a suitable artificial diet that would reduce the cost of rearing and insure a constant supply of food. It is the authors' goal that the results of this study encourage further research into the implementation of *D. terminatus* in the augmentative biological control of pest aphids.

CHAPTER 3
COMPARATIVE QUALITY EVALUATION OF *DIOMUS TERMINATUS* (COLEOPTERA: COCCINELLIDAE) REARED ON *RHOPALOSIPHUM MAIDIS* (HOMOPTERA: APHIDIDAE) AND THOSE REARED ON A SUPPLEMENTARY ARTIFICIAL DIET

Introduction

The high cost of beneficial insects, and concern regarding inconsistent supply and quality are major reasons why many growers choose to follow a chemically based pest management program over a biologically based program (Hale and Elliot, 2003). Many beneficial insectaries raise predatory or parasitic insects in tritrophic systems, where the pest and its host have to be cultured prior to raising the beneficial insects. Artificial diets may make it possible to raise beneficial insects outside of a tritrophic system, which would lower the cost of production and could make biological control more accessible to growers. It has been argued by Singh (1985) and Grenier et al. (1994) that the lack of suitable artificial diets in insect mass production is the greatest barrier to the widespread implementation of beneficial insects and biological control.

The development of an effective artificial diet is a very complicated process involving a thorough understanding of insect behavior, biology and physiology. The insect must first be attracted to the food and then initiate and sustain feeding until sufficient nutrients have been consumed (Moore, 1985). The difficulties in developing an artificial diet are compounded by the fact that some predators depend on complex chemical cues to stimulate prey searching behavior and acceptance. These chemicals must be identified, manufactured, and incorporated into the diet. Even with proper

physical and chemical stimuli, the nutrients in the diet must be qualitatively and quantitatively appropriate to support normal growth, development, fecundity and behavior (Cohen, 1992). Cohen (2003), Moore (1985), and Singh (1985) provide thorough overviews on the various considerations in designing artificial diets. The description of many artificial diets for insects have been compiled by House (1967), House et al. (1971), and Singh (1977).

In our trials, the decision to experiment with artificial diets in the rearing of *Diomus terminatus* (Say) arose out of necessity. We were unable to maintain aphid colonies of *Aphis gossypii* Glover, *Myzus persicae* (Sulzer), *Rhopalosiphum maidis* (Fitch) or *Aphis nerii* Boyer de Fonscolombe, in a greenhouse in the summer of 2002. This was due in part to insufficient cooling, and infestations of natural enemies in the greenhouse aphid colony. Hence, we experimented with alternative foods and artificial diets in order to maintain the *D. terminatus* colony.

Prior to attempting to rear *D. terminatus* on artificial diets, it was necessary first to determine if they consumed alternative foods. In several no-choice tests, we found *D. terminatus* adults survived at least 1 week on a diet consisting solely of whiteflies *Bemesia argentifolii* (Homoptera: Aleyrodidae), red-banded thrips *Selenothrips rubrocinctus* (Giard) (Thysanoptera: Thripidae), bee pollen and eggs of the grain moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Next, trials were performed in order to ascertain that *D. terminatus* did not need a live plant, but would reproduce and survive on excised plant leaves. These behaviors indicated *D. terminatus* had the potential to survive on an artificial diet. Hence, we attempted to rear *D. terminatus* on an artificial diet developed by Entomos®, Inc. (US Patent #6,291,007), which used dried

egg yolk as the protein base. Entomos® had developed this diet to rear the coccinellid *Coleomegilla maculata* (DeGeer), the geocorid *Geocoris punctipes* (Say), and the anthocorid *Orius insidious* (Say).

Although it was evident that *D. terminatus* larvae and adults readily consumed and survived on this diet, there was only minimal fecundity, even when the diet was supplemented with *Ephestia* eggs. In a pretrial experiment, two colony cages that were provided with artificial diet and *Ephestia* eggs laid few or no eggs for four weeks. However, fecundity was restored after only 4 days when the artificial diet was supplemented with the aphid *Rhopalosiphum maidis* (Fitch). Hence, it became clear *D. terminatus* could not be reared on Entomos® artificial diet and *Ephestia* eggs alone. However, the diet might be beneficial as a supplemental food source in times of aphid shortage.

Prior to adopting Entomos® artificial diet in the large scale rearing system of *D. terminatus*, it was necessary to first determine the effects of supplemental artificial diet on insect quality. Trials were performed to determine the effects of supplemental artificial diet under periods of semi-starvation (one aphid per day), as well as the effects of parental consumption of the artificial diet on the quality of the offspring as demonstrated in fecundity, fertility, larval development, adult size and sex ratios.

Materials and Methods

Adult *D. terminatus* were obtained from a laboratory colony at the Mid-Florida Research Station in Apopka, Florida, in Jan. 2001. The species identification was confirmed by Dr. Michael Thomas, of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. The beetles were maintained in Florida Reach-In® environmental growth chambers (Walker et al., 1993) at the University of

Florida, Department of Entomology and Nematology. The growth chambers were programmed to maintain a consistent temperature of 27° C, 60% humidity, and a photoperiod of 16L: 8D h. There were at least two colony cages of each treatment: A diet of the corn leaf aphid, *R. maidis* provided only on sorghum leaves (RM), and *R. maidis* supplemented with Entomos® artificial diet (AD). Approximately 2.5 ml of diet was provided daily, along with *R. maidis* on sorghum leaves, to the AD treatment colony. Uneaten diet was removed from the colony cage every 24 hours.

Experiment 1: Fecundity When Provided a Single Aphid, or a Single Aphid Supplemented with Entomos® Artificial Diet

Pupae were collected from the RM colony cages and when they eclosed, adults were provided *Ephestia* eggs for 10 days. Twenty-four adults were isolated into individual Petri dishes (5 cm x 0.9 cm) and provided either a single *R. maidis* nymph (RM), or a *R. maidis* nymph and a 0.5 cm cube of Entomos® diet (RM+). There were 12 individuals in each treatment and four replicates. All beetles also were supplied with a strip of wax paper (4 cm x 1 cm) and a droplet of free water. A fresh aphid, water, and diet (where appropriate) were provided every 24 hours for 10 days, and the number of eggs laid was recorded daily. Every three days the eggs were collected and isolated from the beetles to determine if egg fertility was affected by the treatments. The beetles that did not lay eggs were dissected to determine gender. Proportional data were analyzed with a test to evaluate two binomial proportions. Daily fecundity data were analyzed with the t-test. Both analyses were performed using Minitab (Minitab Statistical Software, 2003).

Experiment 2: Fecundity as a Function of Larval and Parental Nutrition

The purpose of this study was to determine the effect of larval and adult nutrition on fecundity. Pupae were collected from a single cohort from the two colony treatments, RM and AD, and adult *D. terminatus* were provided *R. maidis* on sorghum leaves (*Sorghum bicolor* var. hybrid grain sorghum SS800) for 10 days to complete the preoviposition period. This insured sufficient time for mating and completion of the preoviposition period. After the 10 days, the beetles were isolated in small, lipped petri dishes (5-cm x 0.9-cm) and provided daily with a diet of either 5 or 10 large *R. maidis* nymphs, a drop of water, and a small strip of wax paper (4-cm x 1-cm) for laying eggs. The number of eggs laid and aphids consumed were recorded daily. Eggs laid directly on the petri dish were marked on the outside of the dish with a wax pencil to insure they were not counted twice. Beetles that did not lay eggs after 10 days were dissected to determine the gender. Data were recorded on the beetles each day for at least 20 days. Every 3 days, *D. terminatus* adults were transferred to a new petri dish to insure fecundity was not negatively impacted by contamination from frass. There were 24 beetles from each colony treatment tested, 12 for each of the two feeding regimes of five or ten aphids. Comparisons were made on the number of eggs laid per female per day using General Linear Model (GLM) and LSmeans procedures in SAS (The SAS System for Windows v8, 2001). All trials occurred in the same growth chamber at 27° C, 60% humidity, and a photoperiod of 16L: 8D.

Experiment 3: Egg Fertility as a Function of Larval and Parental Nutrition

Fertility of the eggs was determined by counting the number of larvae that emerged from the eggs after 3 days. Every 3 days the beetles in the previously described trials (Exp. 1 and 2) were removed from the Petri dishes and the number of eggs in the Petri

dish were counted. Because the eggs laid directly on the Petri dish were not removed in between collections, eggs up to 3 days old were collected. The inside of the Petri dish was misted with water and sealed with Parafilm® to insure sufficient humidity. The dishes were placed in the growth chamber at 27° C and 16L: 8D. The Non-parametric Wilcoxon Signed Rank test was performed on the median percent emergence (Minitab, version 3.1 2002).

Experiment 4: Larval Development as a Function of Parental Nutrition

Previous trials demonstrated that *D. terminatus* larvae are very sensitive to handling and experience high mortality and delayed growth when disturbed by daily measurements of the instars. Pretrials also showed that the larvae often eat their cast skins, making it impossible to determine a molt based on the number of cast skins. Hence, data were collected on preserved larvae with measurements taken on the distance between the semmata (simple eyes) to determine instar, as well as the total body length, according to the procedures described by Hentz and Nuessly (2002).

Single clutches of eggs were collected every 24 hours from isolated females fed at least 15 *R. maidis* per day. These females were maintained in Petri dishes in the Florida Reach-In growth chambers® (Walker et al., 1993) 27° C and 16L: 8D. Aphids were added to each dish of eggs to insure food would be available for the larvae immediately upon emergence. The first day that larvae were seen in the dish was considered day one. Larvae were collected and measured from day one until only prepupae and pupae remained, approximately 7 days later. All the larvae from at least two clutches of eggs in each treatment (RM and AD) were killed, and 25 larvae were randomly collected and measured. Larvae were killed in hot water with a small amount of dish soap as a surfactant, measured, and preserved in 80% ethanol. Measurements were taken at 80x

using a dissecting microscope with an eyepiece scale. Statistical analyses of the data were performed using the procedure for estimating a mixture of distributions using S+ (6.0 professional release 2 © Insightful Corp).

Experiment 5: Cohort Sex Ratios as a Function of Larval Nutrition

To determine the sex ratio of each cohort, all the beetles from three cohorts of the RM treatment (144 beetles) and four cohorts of the AD treatment (123 beetles) were dissected. Pupae were collected from each cohort 10 days after egg deposition. Adults were frozen at -10° C upon eclosion. The gender of the beetle was determined by dissection and identification of the aedeagus or spermatheca. Dissections were made under an 80x-dissecting microscope in 70% ethanol. Data were analyzed with binomial proportions for one and two proportions (Minitab Statistical Software, 2003).

Experiment 6: Adult Size, Determined by the Length and Width of the Elytra and Pronotum, as a Function of Larval Nutrition

To determine if there were any differences in size between the colony treatments or between the genders, all pupae from a single cohort of both treatments were killed, measured and dissected. Data were taken on 39 RM adults and 37 AD adults. The following measurements were taken: width at the base of the pronotum, length across the center of the pronotum, maximum width of the elytra, and elytral length. Measurements were made using an 80x-dissecting microscope with an ocular measuring scale. All dissections were made in 70% ethanol. Data were analyzed using t-test (Minitab Statistical Software, 2003).

Results

Experiment 1: Fecundity When Provided a Single Aphid, or a Single Aphid Supplemented with Entomos® Artificial Diet

The results of this trial showed an increase in the proportion of fertile females when beetles fed a single aphid per day were supplemented with artificial diet (Z=-2.01, P=0.045). Data of the mean fecundity per female were square root transformation to meet assumptions for the t- test. The results from this analysis indicated the average number of eggs per female also improved when supplemented with artificial diet (T=-4.16, P=0.000). These data indicated that both the percentage of females that laid eggs (RM: 53.1% vs. RM+: 76.7%) and the number of eggs laid (RM: 0.46 vs. RM+: 1.00) increased when beetles provided a single aphid per day were supplemented with artificial diet.

Table 3-1. Mean ± SE number of eggs laid per day for *D. terminatus* provided *R. maidis* (RM) daily or a single *R. maidis* supplemented with a 0.5 cm cube of Entomos® artificial diet (RM+)

Treatment	No. Females	No. Fertile Females	Percent Fertile Females	Mean Fecundity
RM	32	17	53.1%	0.46 ± 0.08
RM+	30	23	76.7%	1.00 ± 0.11

Experiment 2: Fecundity as a Function of Larval and Parental Nutrition

All of the females in this trial laid eggs, and the least squares means demonstrated a significant effect on fecundity by aphid consumption. After analyzing the six levels of aphid consumption (4-10 aphids/day), the results showed that the amount of eggs laid were grouped into two categories based on differences in fecundity between levels of aphid consumption. There were no significant differences among 4, 5, and 6 aphids consumed per day (Group 1), however Group 1 showed a significant difference with 7, 8, 9, and 10 aphids consumed per day (Group 2) (T =-6.27, P<0.0001), which are not

significantly different among themselves. Due to the high standard deviation, Group 1 fecundity was not significantly different between the two colony treatments (AD Group 1: 8.23 ± 5.53 and RM Group 1: 7.74 ± 5.61, T= -6.75, P =0.5041) (Figure 3-1). However, there were significant differences in fecundity between colony treatments when higher amounts of aphids were provided (AD Group 2: 10.95 ± 5.92, RM Group 2: 14.13 ± 8.14, T= 2.95, P= 0.0034).

Within the colony treatments, the amount of aphids consumed also effects fertility. AD Group 1 averaged 8.23 ± 5.53 eggs, and AD Group 2 averaged 10.95 ± 5.92, (T= -2.82, P =0.0049), while RM Group 1 averaged 7.74 ± 5.61, and RM Group 2 averaged 14.13 ± 8.1 (T =-7.3056, P <0.001). Additionally, fecundity decreased at a similar rate over time for both colony (RM or AD) treatments (Figure 3-1).

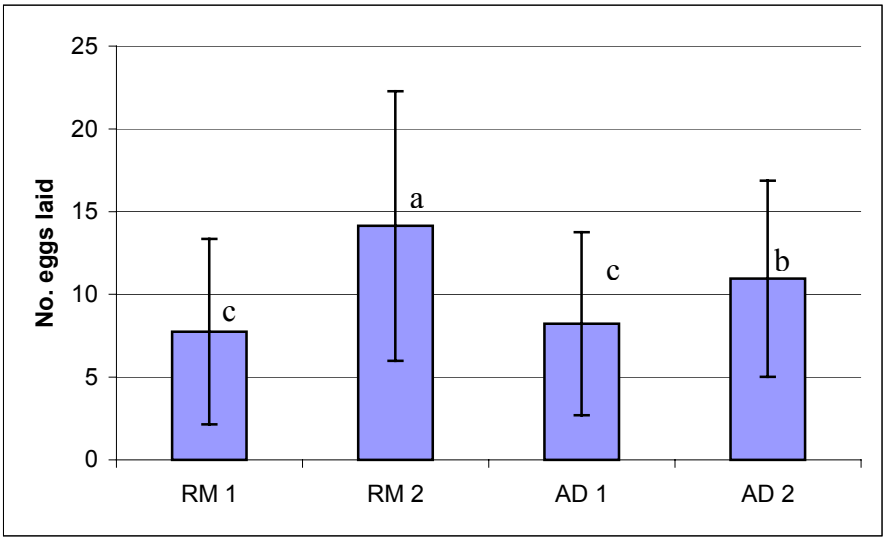

Figure 3-1. *Diomus terminatus* daily fecundity as a function of aphid consumption and larval nutrition. RM treatment were provided aphids only as larvae, AD were supplemented with artificial diet as larvae. Group 1: 4-6 aphids/day, Group 2: 7-10 aphids per day. Different lowercase letters indicated significant differences among the treatments (LSD P<0.05).

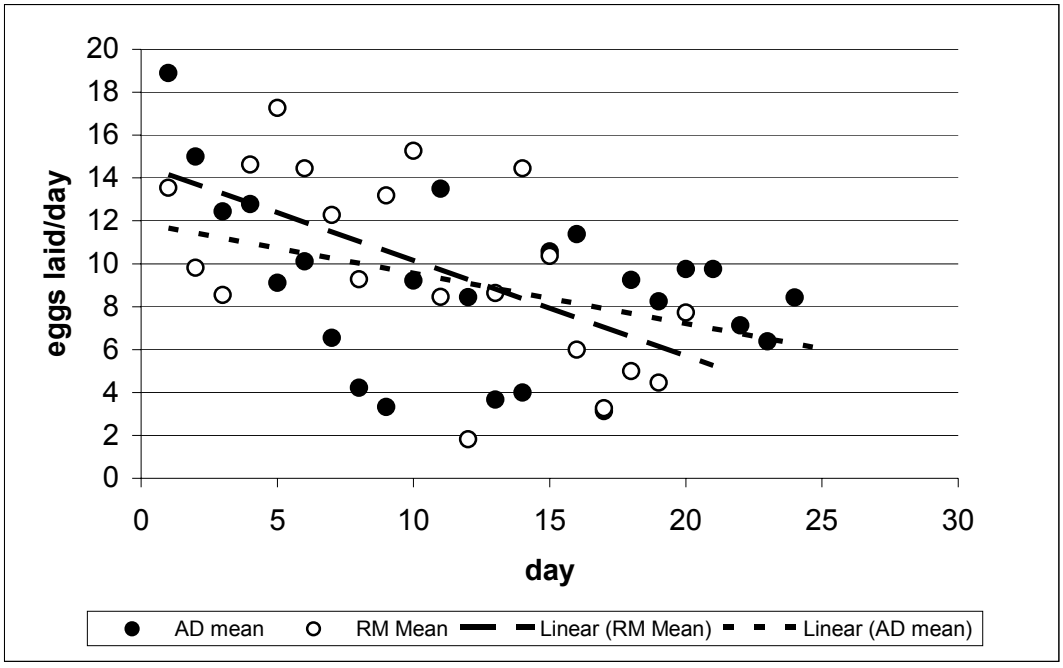

Figure 3-2. Daily fecundity over time for *D. terminatus* provided only aphids as larvae (RM), and beetles provided aphids and supplemental artificial diet as larvae (AD)

Experiment 3: Egg Fertility as a Function of Larval and Parental Nutrition

The median fertility for beetles provided five aphids per day (88.2 %) was comparable to the median fertility when provided 10 aphids per day (84.0%). However, egg fertility was significantly lower (69.2%), when adults were provided only one aphid per day. The median emergence improved to a level similar to the well-fed aphids (80.0%) for *D. terminatus* that were provided one aphid plus artificial diet (Table 3-2).

Table 3-2. Median egg fertility for *D. terminatus* adults of four nutritional treatments[a]

Treatment	N	Estimated Median	95% Confidence interval
5 aphid	70	0.882	(0.850, 0.912)
10 aphid	39	0.840	(0.768, 0.907)
1 aphid	17	0.692	(0.500, 0.875)
1 aphid +diet	47	0.800	(0.700, 0.900)

[a]The Non-parametric Wilcoxon Signed Rank test was performed on the median percent emergence

Experiment 4: Larval Development as a Function of Parental Nutrition

Although the eggs were collected over a 24-hour period, most larvae emerged over a 48-hour period 3 days after they were laid. *Diomus terminatus* larvae progressed through four instars in both of the treatments. This was demonstrated by the four peaks found in the histogram of measurements of the distance between the semmata (Figure 3-3). To emphasize the distribution, presence, and location of gaps, the density histograms were scaled have their so that the sum of the bar heights times the bar widths will sum to one. These components seem to have the same means and roughly the same variance, suggesting that they are structural components of the insect and not an artifact of the treatments. Body size did not correlate directly to instar, in that each instar did not have a specific easily discernable size distribution as was see with eye width. However, the body length histograms of both treatments clearly demonstrated bimodality (Figure 3-4).

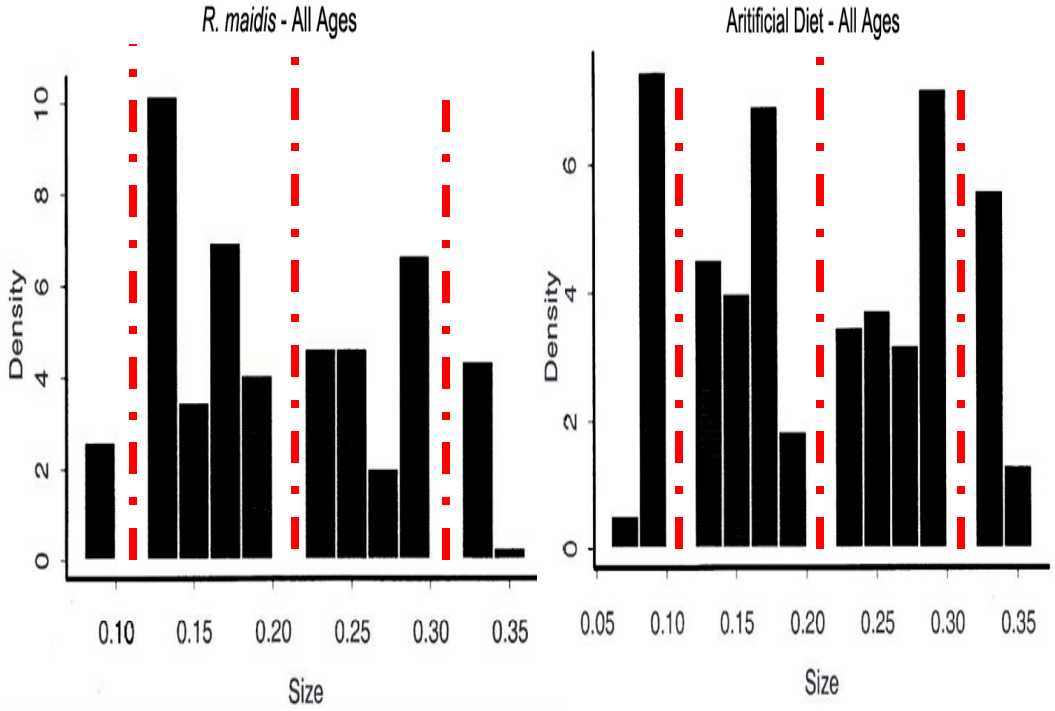

Figure 3-3. Larval development of *D. terminatus* based on distance between eyes. The four groupings, separated by the dashed line indicate four instars.

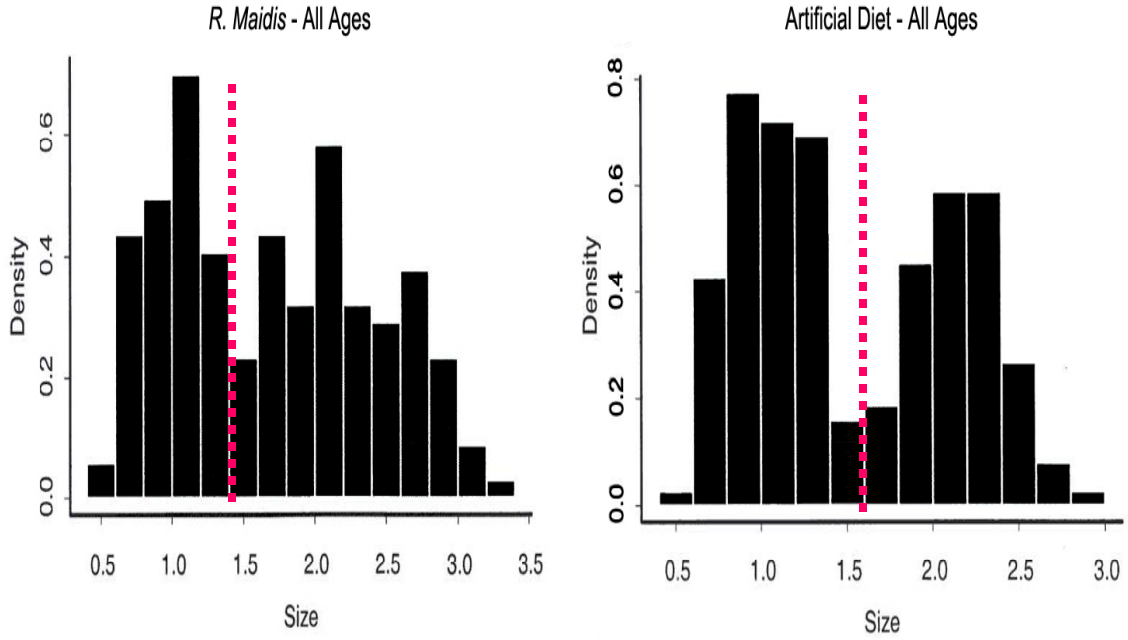

Figure 3-4. Body length measurements of larval *D. terminatus*. The dashed lines indicate the bimodal distribution.

Experiment 5: Cohort Sex Ratios as a Function of Larval Nutrition

The pooled ratio of females to males that survive from egg to adulthood in the RM treatment (1.00: 0.095) was not significantly different from the AD treatment (1.00: 1.05) (Z= -0.03, P=0.978) (Table 3-3). Within each cohort, the sex ratio was not significantly different from 1:1, except for one cohort, AD 4, which is marginally significantly different (P= 0.048).

Table3-3. Sex Ratios from seven cohorts of *D. terminatus*

Gender	RM 1	RM 2	RM 3	AD 1	AD 2	AD 3	AD 4	RM total	AD total
Female	33	12	25	18	15	9	21	70	63
Male	43	11	20	21	8	15	16	74	60
Ratio (F:M)	0.77: 1.00	1:00: 1.09	1.00 1.25	1.00: 0.86	2.25: 1.00	0.60: 1.00	0.81: 1.00	1.00: 0.95	1.00: 1.05

RM treatment were provided aphids only as larvae, AD were supplemented with artificial diet as larvae.

Experiment 6: Adult Size, Determined by the Length and Width of the Elytra and Pronotum, as a Function of Larval Nutrition

Measurements were made on 17 AD females and 18 RM females, and 21 AD males and 22 RM males (Table 3-6). The only sexual dimorphism was observed in the elytra length of the AD treatment. Females averaged a wider elytra length, 1.39 ± 0.01 mm while males averaged 1.31± 0.02 mm (T= 2.49, P= 0.018). In the RM treatment size differences in the genders were observed in the elytra length (T= 2.56, P= 0.015) and elytra width (T= 3.45, P= 0.001). However, between the treatments, the only significant difference was found in the elytra width of the males. Males in the AD treatment had significantly wider elytra, 1.20 ± 0.01 mm, than the males in the RM treatment 1.23 ± 0.01 mm(T= 2.17, P= 0.036).

Table 3-4. Measurements (mm) of the mean ± SE length width and width of the elytra and pronotum of *D. terminatus* when provided *R. maidis* only (RM) and when supplemented with artificial diet (AD)

Treatment	N	Elytra width	Elytra length	Pronotum width	Pronotum length
RM female	18	1.23 ± 0.01	1.38 ± 0.02	0.91 ± 0.01	0.36 ± 0.01
RM male	22	1.17 ± 0.01	1.30 ± 0.02	0.88 ± 0.01	0.33 ± 0.01
AD female	17	1.23 ± 0.01	1.39 ± 0.01	0.92 ± 0.01	0.37 ± 0.01
AD male	21	1.20 ± 0.01	1.31 ± 0.02	0.90 ± 0.01	0.35 ± 0.01

Discussion and Conclusions

For the purpose of rearing *D. terminatus*, the research showed that Entomos® artificial diet can be used as a supplemental food source when there is an inadequate amount of aphids were available. The results of this study demonstrated that egg fertility improved from 69.2% to 80.0% under semi-starvation conditions, when the daily supply of a single aphid was supplemented with artificial diet. Additionally, a higher percentage of females were fertile when supplemented with artificial diet under semi-starvation conditions, 76.6% compared to 53.1%. This percentage increased even further in

experiment 2 when 100% of the females were fertile when provided at least 5 aphids per day. However, this diet supported the production of fewer eggs compared to an abundant supply of aphids, as demonstrated in experiment 2. There were no significant effects on sex ratios due to the colony treatments, and only a slight difference in the elytra width of the males in between treatments. The effects of the feeding regimes on larval development were less clear. It is unusual to have two distinct ranges in body size although there were four instars, as determined by distance between the semmata. A more detailed investigation into these data are necessary in order to come to a conclusion.

There are often legitimate concerns regarding insect quality in mass-production, and especially when artificial diet is used. This does not mean mass-production and artificial diets do not have value, and should not be pursued. Although it was not directly tested, it can be inferred that under reduced aphid availability, artificial diet could help reduce cannibalism. Artificial diet also would insure a reliable food source for biological control agents in shipment, or could be used to maintain field populations of beneficial insects when prey is scarce.

While exposure to artificial diet as larvae correlated to reduced fecundity, it has not been determined whether these differences would correlate to any decrease in quality of the insect as a predator when released into the field. Should the efficacy of a beneficial insect be reduced due to the rearing methods, the lower cost of mass-produced insects, especially those reared with artificial diet would significantly lower the cost to the grower, and may offset any increase in cost due to the number of insects that need to be released.

CHAPTER 4
SUMMARY AND CONCLUSIONS

The goals of this research were threefold. The first was to develop a better understanding of the basic biology of *D. terminatus*. The second was to develop and refine a novel rearing system for mass-production of this insect. The third objective was to evaluate the effects of supplementing Entomos® artificial diet to a diet of the corn leaf aphid, *R. maidis.*

Successful rearing of *D. terminatus* was achieved by a system that grouped the insects by developmental state, eggs, larvae, and adult. By placing adults in separate containers cannibalism was reduced and feeding, harvesting, and planning was simplified. Adults were isolated in a container and given ample food (*R. maidis* or *R. maidis* plus artificial diet) and water, as well as wax paper strips and Kimwipes® for egg deposition. Adults were then removed after 5 days and transferred to a new container. As the eggs hatched, food was provided according to the age of the larvae, with older larvae provided more food than younger ones. In 10 days, the majority of the eggs had hatched and the larvae had pupated on the Kimwipe®. Harvesting new adults was simply a matter of aspirating them as they eclosed, or by collecting them using phototropism and a light trap.

Experimental trails were performed on cohorts of *D. terminatus* reared using two dietary regimes. One group was fed solely *R. maidis*, while the other was given *R. maidis* supplemented with Entomos® artificial diet. Significant differences in fecundity and fertility were found between the two nutritional treatments at higher levels of aphid

consumption. There were no significant differences between the sex ratios of the cohorts, and only a slight difference between the elytra width of the males between the colony treatments.

Results of this study demonstrated that *D. terminatus* consumed at least 10 *R. maidis* per day, but could survive on as little as a single aphid per day for at least 10 days. The number of eggs laid per day increased with aphid consumption. Beetles provided an average of only one aphid per day laid few eggs, however fecundity and percentage of fertile females increased when supplemented with artificial diet (Table 3-1). The highest fecundity was observed in beetles that consumed 7-10 aphids per day and had not been exposed to artificial diet as larvae. Egg fertility was the lowest when provided a single aphid per day but increased to levels similar to well fed aphids when supplemented with artificial diet.

When compared to other biological control agents, lady beetles are relatively well known to the public. This might make incorporating *D. terminatus* into an insect pest management program easier than it would be for other beneficial organisms used against aphids, such as fungi and parasitic wasps. Additionally, the small size (2 mm adults, 1 mm larvae) is also a benefit as they are less obvious to the end customer. Because *D. terminatus* is native to the Eastern and Midwest United States, it is well suited to the environmental conditions and pest aphids found in Florida. Additionally, there are fewer ecological risks associated with the release of native predators compared to non-indigenous organisms.

The research performed in these experiments is just one step in evaluating the potential of *D. terminatus* as an augmentative biological control agent for aphids. With

the development of an effective mass-rearing method, future trials can be conducted on the efficacy of this predator in greenhouse and field settings. These trials will entail quantifying searching behavior, prey consumption, and reproduction and development of *D. terminatus* in the field. These data could then be used to develop an IPM strategy incorporating release rates of the predator at different pest density levels. Additional lab work should focus on determining the daily satiation limit, and total consumption for the beetles.

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BIOGRAPHICAL SKETCH

My interest in biological control began early in my life when my mother explained how the "good bugs" (lady beetles) were eating the "bad bugs" (aphids) on the hydrangea bushes in front of our Buffalo, NY, home. However, my first direct involvement with biological control and insect rearing did not occur until my sophomore at the University of Arizona. For the next four years, I worked at the university's Beneficial Insect Rearing Facility raising the parasitoids *Encarsia formosa* and *Eretmocerus spp*., to investigate whitefly biological control in cotton fields. In 1999, I accepted a position at Koppert Biological Systems, Inc., in Romulus, Michigan, to develop a mass rearing system for *Tamarixia triozae*, a parasitic wasp of the potato/tomato psyllid, *Paratrioza cockcerelli*. My experiences working in biological control for both the state and commercial industry have greatly expanded my appreciation for both sectors. I foresee greater cooperation between these two sectors as the popularity of biocontrol continues to increase.

I am grateful to have had the opportunity to work at the University of Florida under the guidance of my generous and knowledgeable advisor and committee. My insect rearing skills and knowledge of biological control have strengthened through increased scientific understanding of insects and experimental testing. The coursework, conversations with professors, students and staff, and new experiences with predators and artificial diets have expanded both the breadth and depth of my knowledge of the field.