BIOLOGY OF THE MEALYBUG PARASITOID, *ANAGYRUS LOECKI*, AND ITS POTENTIAL AS A BIOLOGICAL CONTROL AGENT OF THE MADEIRA MEALYBUG, *PHENACOCCUS MADEIRENSIS*

by

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(Under the Direction of S. Kristine Braman)

ABSTRACT

Anagyrus loecki Noyes & Menezes (Hymenoptera: Encyrtidae) is a parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), the most important mealybug pest of greenhouse ornamental production in Georgia. This doctoral dissertation evaluated the potential of *A. loecki* as a biological control agent of *P. madeirensis* through studies on three aspects of *A. loecki* biology:

1) the interactive effects of temperature, mating status and food supplements on the life history of *A. loecki*;
2) the preference and suitability of different mealybug developmental stages for the development and reproduction of *A. loecki*; and
3) the functional and reproductive responses of *A. loecki* to varying host densities.

Anagyrus loecki is an arrhenotokous parasitoid and has an average lifetime fecundity of 78 progeny. The developmental rate of the mealybug parasitoid increased with temperature between 15 and 30^{o}C. The lower development threshold of female parasitoids was estimated to be 11^{o}C and the thermal constant was 227 degree-days. The upper developmental threshold

appeared to be above 30°C. The survival rate of the parasitoid larvae was above 94%. Provision of diluted honey significantly extended the longevity of *A. loecki*, especially at lower temperatures.

A study of foraging behavior suggested that *A. loecki* was able to parasitize and develop in all developmental stages of *P. madeirensis*. Third-instar immatures and pre-reproductive adult mealybugs were the most preferred and suitable host stages. These host stages were able to support the development of a higher number of progeny, a female-biased sex ratio, the shortest developmental time and the highest survival rate.

Anagyrus loecki exhibited a type II functional response, meaning that the parasitism rate decreased exponentially with increase in *P. madeirensis* density. The number of progeny was significantly increased with host density. Based on the prediction by theoretical models, *A. loecki* is not expected to provide sustainable control of *P. madeirensis*. Such prediction may not be accurate because it was based on biased results created in artificial experimental conditions.

The results of this dissertation research suggested that *A. loecki* has the potential to be an effective biological control agent of *P. madeirensis* in greenhouse ornamental production.

INDEX WORDS: Biological control, Greenhouse, Life history, Host selection, Functional response, Numerical response

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DEDICATION

This dissertation is dedicated to Dr. Ronald Dale Oetting, for his 37 (and counting) years of contribution to the science of Entomology, and as my teacher, mentor and friend.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

This doctoral dissertation research evaluates the potential of a mealybug parasitoid, *Anagyrus loecki* (Hymenoptera: Encyrtidae), as a biological control agent of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudoccocidae), through studies on the biological and ecological interactions between the parasitoid and its host. *Phenacoccus madeirensis* is the most damaging pest of greenhouse ornamental production in the southeastern Unites States. The economic importance of *P. madeirensis* and the goal of providing a feasible alternative to conventional management tactics necessitate this research. This dissertation research also provides novel knowledge to the studies of parasitoid life history, host-parasitoid interactions and parasitoid ecology in general.

In this chapter, I will first briefly review the implementation of integrated pest management in ornamental production. This is followed by a discussion on the biology of the Madeira mealybug and *A. loecki*, and a brief introduction to the objectives of individual studies.

THE ECONOMY OF ORNAMENTAL PRODUCTION

Nursery and greenhouse ornamental crops are one of the most important agricultural commodities in the United States. According to the 2002 Census of Agriculture (USDA-NASS 2002), the gross earnings of ornamental and sod production in the United States totaled $4.8 billion, an increase from $ 3.9 billion in the year 1997. Among the 36 states surveyed by the USDA-NASS, the top five producing states of floricultural crops are California ($985 million), Florida ($803 million), Michigan ($323 million), Texas ($ 285 million) and New York ($ 171 million).

In the year 2002, growers in Georgia earned a total of $ 315 million from sales of nursery and greenhouse crops, floricultural crops and sod, and ranked Georgia 13th in sale values nationwide (USDA-NASS 2002). Nursery and greenhouse crops ranked fourth in total sale

values within the state of Georgia, behind poultry products, productions of vegetables, melon, potatoes and sweet potatoes, and cotton. This value represented a 44% increase from the sale values of $219 million in the year 1997.

INTEGRATED PEST MANAGEMENT IN GREENHOUSES

Insects and mites inflict significant damage and losses in ornamental production. In Georgia, the most important insect pests of ornamental production, and their costs of control and damage in parentheses, include scale insects and mealybugs ($ 71 million), mites ($ 56 million), aphids ($ 15 million), whiteflies ($ 13 million), and thrips ($ 12 million) (Oetting et al. 2002). Other pests that caused significant losses to ornamental growers in Georgia are caterpillars, slugs and snails, beetles, lace bugs, spittle bugs, fungus gnats, leafminers and others.

Insect pest management can make up a significant portion of a grower's operational budget. Detailed surveys on pest management pattern of ornamental growers have not been conducted in Georgia. In a survey of 221 Florida ornamental growers, 20% of the respondents spent 16-20% of their budgets on pest management (including labor, equipment and materials) (Hodges et al. 1998). In the same survey, 95% of surveyed Florida ornamental growers used insecticides and miticides. The most commonly used insecticides include acephate (used by 59% of respondents), diazinon (43%) and avermectin (43%). Many surveyed growers indicated that they used more than one insecticide. Only 15% of the respondents employed biological control tactics. Results of the survey suggested that chemical control tactics are the principal pest management tools in the greenhouses of Florida and the southeastern United States.

Chemical control is just one of the many facets of Integrated Pest Management (IPM). Integrated pest management incorporates multiple pest management practices, including biological, chemical, cultural, physical and regulatory. The major goal of IPM is to reduce

pesticide use while maintaining pest population below an economically damaging level. The development of IPM as a discipline started as early as 1939 with a call for discriminative use of insecticides and combination of chemical and biological controls by Hoskins et al. (1939) (cited by Kogan 1998). It takes more than 30 years for the term 'integrated pest management' to be accepted by the scientific community (Kogan 1998). The trend toward more widespread practices of IPM was the results of public concerns for environment and food safety, pesticide resistance and secondary pest outbreaks. Although the adoption of IPM is sometimes constraints by many perceptive, technological and political challenges (Jeger 2000), the movement toward a more integrated approach of pest population management is gaining momentum in both developed and developing countries. In 1993, a joint announcement by the United States Department of Agriculture, the Environmental Protection Agency and the Food and Drug Administration called for the implementation of IPM on 75% of the cropland in the United States (USDA-CSREES 1996).

About 5% of the greenhouses worldwide are currently under IPM, and the number could potentially increase to 20% by 2010 (van Lenteren 2000). One of the main constraints in adopting IPM in greenhouses is the low tolerance for pest damage (van Lenteren and Woets 1988, van Lenteren 2000). If chemical control is more effective in protecting the vegetable or ornamental crops from any pest damage, the growers will continue to use pesticides as the main, and in some cases the only, pest management tool. In addition, chemical control is relatively simple and inexpensive to implement. Insecticide represents only 1% of total cost of greenhouse ornamental production (Parrella et al. 1999). The cost of pest management may increase substantially if a biological control program is implemented. Chemical and biological control are inherently antagonistic. The adoption of IPM practices would require modifications in

conventional chemical control strategies to reduce the disruptive effects of insecticide applications to biological control agents.

There are additional challenges to the implementation of IPM on greenhouse ornamental crops (van Lenteren 2000). First, many different species and cultivars of ornamental plants are grown within the same greenhouse in many operations. The diversity of crops requires different production practices and thus complicates the implementation of biological or cultural controls. Second, each ornamental crop has several important or minor pests and each pest species requires a specific management strategy (Van Driesche and Heinz 2004). As a result, the growers need to develop a network of compatible control strategies for these pests. The third factor slowing the adoption of IPM in greenhouse ornamental production is that the tolerance for pest damage in ornamentals is lower than that in vegetables. Osborne et al. (1994) suggested that while actual damage is often less than 15%, the cosmetic damage to any part of the ornamental plant may render it unmarketable. Finally, more pesticides are available for use on ornamental plants than vegetables. Thus, the ornamental growers have more choices in chemical control and less incentive in adopting biological and cultural controls.

There are generally three categories of biological control: classical, augmentative and conservation. Classical or introduction biological control involves the importation of natural enemies from the native distribution range of an exotic pest for the purpose of managing the exotic pest populations. Augmentative biological control is the release of biological control agents either periodically without the expectation of building a self-reproducing natural enemy population (inundative), or a single release with the control exerted by the progeny of the small number of originally released biological control agents (inoculative). Conservation biological control involves the modifications of physical environment, production practices and pest

management tools, and/or the provisioning of food sources to enhance the longevity and efficacy of the biological control agents.

Augmentative biological control is by far the most commonly practiced type of biological control in greenhouse ornamental production. Augmentative releases of biological control agents have achieved successful controls of several pest species in greenhouse ornamental and vegetable productions. *Encarsia formosa* (Hymenoptera: Aphelinidae) is used extensively to control greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), on greenhouse vegetables. *Encarsia formosa* is used to a lesser extent on ornamental crops than on vegetable crops (Hoddle et al. 1998). Control of the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, on poinsettias was achieved by using *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) (formerly known as *Eretmocerus* sp. nr. *californicus*). The beetle *Delphastus pusillus* LeConte (Coleoptera: Coccinellidae) avoids feeding on parasitized whiteflies and thus is compatible with the use of aphelinid parasitoids (Hoelmer et al. 1994, Heinz & Nelson 1996). Management of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), on vegetable and ornamental crops using predatory mites, such as *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) (both Acari: Phytoseiidae), has been successful. One of the most widely used biological control agents against the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is the predatory mite, *Neoseiulus cucumeris* (Oude.) (Acari: Phytoseiidae).

The selection of potential biological control agents is the first step in designing an augmentative biological control program. Van Lenteren and Woets (1988) proposed a step-wise natural enemy screening scheme for seasonal inoculative biological control programs. Step one assesses whether the candidate species has any obvious negative aspects, for example

hyperparasitism, developmental failure, or low searching efficiency, which would lower the species' effectiveness. Step two tests the ability of the candidate biological control agent to attack the pest on the crop and to develop successfully under the climatic conditions at the site. Laboratory or field experiments aimed at determining the rate of population increase or kill potential of the candidate natural enemy are to be completed before step three. Step three screens out those that have growth rates lower than those of the target pest population (when the pest population is reproducing in the presence of the natural enemy). A failure in any step results in the elimination of the candidate natural enemy from further consideration. Van Lenteren and Woets (1988) also suggested a list of criteria for pre-introductory evaluation of candidate biological control agents for greenhouses. A selected candidate natural enemy should possess the following characteristics:

1) For a successful inoculative biological control program, the selected natural enemy should be able to complete development in or on the target pest. The life cycle of the candidate natural enemy should also be synchronous with that of the target pest to prevent cyclical outbreaks. Reintroduction in the subsequent pest generation is required for inundative releases because the natural enemy population will not be established permanently.
2) The selected natural enemy should be able to develop, reproduce and disperse in the climatic conditions of the intended release sites.
3) There should not be any determined non-target effects of the selected natural enemy on other beneficial or non-pest organisms.
4) The selected natural enemy should be easy to rear in order to keep the production cost low.

5) The potential of population increase of the selected natural enemy should be equal to or greater than that of the target pest. Candidate natural enemies with medium kill rate may be used in inundative releases if the efficiency of these natural enemies can be enhanced by increasing the number of individuals released or by selecting plant varieties that are either resistant to the target pest or synergistic to the searching process of the natural enemy (Van Driesche and Bellows 1996).
6) The selected natural enemy should be able to locate the target pests effectively and reduce the pest populations before they reach economic damaging levels.

Van Lenteren (2000) listed seven reasons why growers prefer biological control over chemical control: 1) absence of phytotoxicity, 2) release of natural enemies takes less time and is more pleasant than insecticide applications, 3) biological control requires less time committed for monitoring, 4) effective against pesticide resistant pests, 5) no re-entry interval (REI) thus allowing continuous operation, 6) control is sustainable, and 7) biological control is appreciated and reduced pesticide use is demanded by general public. Proponents of biological control could use some of these advantages to make a strong case for research and applications of biological control in greenhouses. Parrella et al. (2004) concluded that biological control will play a greater role in greenhouse vegetable and ornamental productions in the future. They also suggested several trends for the future direction of biological control in protected cultures. First, more research into the biology and application of natural enemies is needed to improve predictability and reliability of the biological control program, which at the same time maintaining simplicity and low cost of the system. Second, a stronger collaboration between researchers, insectaries and growers is needed to promote the adoption of biological control. In addition, researchers should understand the production systems and promote compatible biological control

approaches. Finally, research into the integration of biological and chemical control is essential to the adoption of biological control in greenhouse ornamental production.

THE MADEIRA MEALYBUG, *Phenacoccus madeirensis*

Biology and pest status of *Phenacoccus madeirensis*

The Madeira mealybug, *Phenacoccus madeirensis* Green, is the most important mealybug species in the ornamental production of Georgia. Other important mealybug species include the citrus mealybug, *Planococcus citri* (Risso), the longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti), and the root mealybugs, *Rhizoecus* spp. The striped mealybug, *Ferrisia virgata* (Cockerell), and the obscure mealybug, *Pseudococcus viburni* (Signoret) are occasional pests on woody ornamentals and foliage crops.

Phenacoccus madeirensis was first described from specimens collected on the Madeira Island (Green 1923). *Phenacoccus madeirensis* is often misidentified as the Mexican mealybug, *Phenacoccus gossypii* Townsend & Cockerell. The misidentifications have often been based on the illustrations and descriptions by Myers (1928), Ferris (1950) and McKenzie (1967). McKenzie (1967), for example, provided detail descriptions of *P. gossypii* but did not list *P. madeirensis*. A review of *P. gossypii* and its related species by Williams (1987) has clarified the taxonomic confusions between *P. madeirensis* and *P. gossypii*. The two closely related species differ in that *P. gossypii* possesses numerous multilocular pores on the median dorsal areas of the thorax, which is a morphological characteristic absent in *P. madeirensis* (Williams 1987). My examination of mealybug specimens deposited at the University of Georgia Museum of Natural History suggested that specimens previously collected in Georgia and identified as *P. gossypii* were in fact *P. madeirensis*.

The Mexican mealybug is known to occur in Florida, Texas and Mexico. In contrast, the Madeira mealybug has a cosmopolitan distribution and feeds on more than 40 plant families, many of which are economically important crops and ornamental plants (Ben-Dov 1994). Williams (1987) and Williams and Granara de Willink (1992) suggested that the worldwide distribution of *P. madeirensis* might be the result of introductions from tropical America.

Chong et al. (2003, 2004) have studied the life history of *P. madeirensis*. A female Madeira mealybug produced more than 250 eggs in a week at a constant temperature of 25^{o}C (Chong et al. 2003). The highest fecundity occurred at 20^{o}C where an average of 490 eggs were produced by the mealybugs in two weeks. These eggs hatched into equal numbers of males and females. The female mealybugs completed development in 66 days at 15^{o}C, 46 days at 20^{o}C and 30 days at 25^{o}C. The duration of development of males was three to nine days longer than that of the females. Although the experiment using excised chrysanthemum leaves failed to yield successful development at 30^{o}C (Chong et al. 2003), the whole-plant experiment indicated a developmental duration of 21 days at 30^{o}C (Chong et al. 2004). The developmental times of *P. madeirensis* reported by Chong et al. (2003, 2004) were similar to those reported by Sinacori (1995) in Italy. Overall, more than 75% of the mealybugs completed development to adulthood in laboratory conditions (Chong et al. 2003). The high survival rate and reproductive capacity of *P. madeirensis* allows the mealybug population to reach an economic damaging level within a relatively short period of time. Successful management of *P. madeirensis* in greenhouse ornamental production requires early detection and control of the mealybug population.

The management of mealybugs in greenhouse ornamental production relies heavily on chemical control. Some of the most effective chemicals against the Madeira mealybug include organophosphates, pyrethroids, insect growth regulators, insecticidal soaps and horticultural oils

(Townsend et al. 2000). Successful control of *P. madeirensis* requires sufficient spray coverage and repeated applications targeting immature mealybugs. With the phasing out of organophosphates under the Food Quality Protection Act (FQPA) and the rising environmental and economic concerns against chemical management practices, biological control has become an attractive alternative for managing mealybug populations in greenhouses. Currently, no biological control agent is recommended for the control of *P. madeirensis*. Several natural enemies, for example the parasitoids *Leptomastix dactylopii* Howard and *Leptomastidea abnormis* (Girault) (both Hymenoptera: Encyrtidae), and the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), are available for the control of citrus mealybug. The efficacy of these citrus mealybug natural enemies against the Madeira mealybug has not been tested. There is a need to identify and evaluate novel biological control agents against the Madeira mealybug. The availability of biological control agents specifically targeting *P. madeirensis* will be beneficial to the ornamental growers by providing a feasible alternative to chemical control. Efforts are currently underway in both Florida and Georgia to identify and evaluate potential biological control agents against the Madeira mealybug.

Natural Enemies of *Phenacoccus madeirensis*

Little is known about the natural enemies of *P. madeirensis*. By contrast, the natural enemies of other economically important mealybug species such as *Planococcus* and *Pseudococcus* species are better known. Adding to the lack of detailed studies and surveys of natural enemies of *P. madeirensis*, the taxonomic confusion surrounding this mealybug species compounded the difficulty in identifying the appropriate mealybug-natural enemy relationships. In the following discussions on the reported natural enemies of *P. madeirensis*, I will also include the natural enemies of *P. gossypii*, and *Phenacoccus grenadensis* Green & Laing and

Phenacoccus harbisoni Peterson, both of which were synonymized with *P. madeirensis* (Williams 1987). Table 1 lists the known predators and parasitoids of these mealybug species. The poor record of predators and parasitoids of *P. madeirensis* is reflective of the lack of sampling and survey efforts. Sources of primary and secondary references include Gordon (1985), Hodek and Honěk (1996), Noyes and Hayat (1994), Miller et al. (2004), the ScaleNet (Ben-Dov et al. 2004), the Universal Chalcidoidea Database (Noyes 2003), and the BioSystematic Database of World Diptera (Thompson 2004).

Predators are some of the most commonly used biological control agents and play an important role in regulating insect populations. Main predators of mealybugs include members of the orders Coleoptera. Beetles of the subfamily Scymninae and Chilocorinae are some of the most important scale insect predators. Mealybug-feeding coccinellids are reported in the genera *Cryptolaemus*, *Diomus*, *Exochomus*, *Hyperaspis*, *Nephus*, *Sidis* and *Parasidis* (Hodek and Honěk 1996). Two species (*C. montrouzieri* and *Diomus austrinus* Gordon) have shown promise as biological control agents of *P. madeirensis* in protected cultures (L. S. Osborne, personal communication). *Cryptolaemus montrouzieri* is commercially available for the control of mealybugs. This species was introduced from Australia into the United States in 1890s and 1930s for control of the citrus mealybug, but it also feeds on mealybugs of the genus *Pseudococcus*, *Phenacoccus* and *Ferrisia* (Gordon 1985). Currently, natural populations of *C. montrouzieri* are established in California and in central and southern Florida. *Diomus* species feed on many species of aphids, psyllids, mealybugs and whiteflies. *Diomus austrinus* is a native coccinellid of southern Florida and it has been shown to develop and survive on eggs of *P. madeirensis* and *P. citri* (Chong et al. 2005).

The lacewings (Neuroptera: Chrysopidae and Hemerobiidae) have been reported as predators of mealybugs of the genus *Antonina*, *Ferrisia*, *Maconellicoccus*, *Nipaecoccus*, *Phenacoccus*, *Planococcus*, *Pseudococcus* and *Rastrococcus* (Miller et al. 2004). Two green lacewing species, *Chrysoperla carnea* (Stephens) and *Chrysoperla rufilabris* (Burmeister), are sold commercially as biological control agents of aphids, whiteflies and mealybugs in greenhouses. These two green lacewing species are not reported as predators of *P. madeirensis* or *P. gossypii* but because of their polyphagous nature we expect them to be candidate biological control agents of the two mealybug species. The efficacy of these commercially available predators has not been tested in greenhouses against *P. madeirensis*.

Dipteran predators of *P. madeirensis* and *P. gossypii* are rarely reported. *Toxomerus marginata* Macquart reportedly feeding on *P. gossypii* (possibly a misidentification of *P. madeirensis*; Heming 1936). However, the validity of the syrphid species name cannot be confirmed with Dipteran database (Thompson 2004), and the most similar valid name is *Toxomerus marginatus* (Say). The family Cecidomyiidae contains some of the most commonly encountered dipteran predators of mealybugs. Harris (1968) recognized 10 mealybug-feeding cecidomyiid genera in the world: *Arthrocnodax*, *Coccodiplosis*, *Dicrodiplosis*, *Ghesquierinia*, *Lestodiplosis*, *Megommata*, *Nipponodiplosis*, *Triommata*, *Trisopsis*, and *Vincentodiplosis*. However, no cecidomyiid species was reported as a predator of *P. madeirensis* or *P. gossypii*.

Five families of parasitic Hymenoptera are known to include primary parasitoids of mealybugs: Encyrtidae (109 genera), Pteromalidae (8 genera), Aphelinidae (1 genus: *Coccophagus*), Eulophidae (1 genus: *Aprostocetus*) and Platygastridae (1 genus: *Allotropa*). Members of the family Encyrtidae (tribe Anagyrini) are the most important parasitoids used in mealybug biological control programs around the world (Noyes and Hayat 1994). The

introductions of *Anagyrus* (= *Apoanagyrus*) *lopezi* (De Santis) against the African cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, and *Gyranusoidea tebygi* Noyes against the mango mealybug, *Rastrococcus invadens* Williams, have saved the livelihood of thousands of subsistence farmers in Africa. Table 1 presents 34 species in the family Encyrtidae as primary parasitoids of *P. madeirensis* and/or *P. gossypii*. *Acerophagus coccois* Smith is a polyphagous species attacking a wide range of mealybug species. *Acerophagus coccois* completed development in *P. madeirensis*, the South American cassava mealybug, *Phenacoccus herreni* Cox & Williams, and the striped mealybug, *Ferrisia virgata* Cockerell (Dorn et al. 2001). In Georgia, *A. coccois* is the most important parasitoid species attacking the mealybug *Oracella acuta* (Lobdell) in pine orchards (Sun et al. 2004). *Coccidoxenoides perminutus* [= *C. peregrinus* (Timberlake) also = *Pauridia peregrina* Timberlake) Girault was first introduced into California as a biological control agent of *P. citri* and *Planococcus ficus* (Signoret) (Bartlett 1978), but is now widely distributed in the Unites States. *Leptomastix dactylopii* (Howard), *Anagyrus pseudococci* (Girault) and *Leptomastidea abnormis* (Girault) are commercially available and are frequently used against *P. citri* in protected cultures. However, the efficacy of these parasitoids against *P. madeirensis* has not been studied.

THE MEALYBUG PARASITOID *Anagyrus loecki*

Anagyrus loecki Noyes & Menezes has been identified as a potential biological control agent of *P. madeirensis* by Dr. Lance Osborne of the University of Florida, Mid-Florida Research and Education Center, Apopka, FL. This is a small wasp (female: 1.5-1.8 mm in length; male: 1.0-1.1 mm in length) displaying sexual dimorphism. The female body is orange, with the antennae largely blackish but white on the distal one-third. Males are entirely dark brown or black.

Anagyrus loecki was described from specimens collected in Costa Rica (Noyes 2000). The known distribution of this species also includes Saint Kitts and Nevis, Mexico, Florida and Texas. However, Noyes (2000) questioned the accuracy of the records of *A. loecki* in Texas. The paratypes supposedly from Texas were part of a series of *Anagyrus sinope* Noyes and Menezes under laboratory culture in Trinidad. The Trinidad culture was established with parasitoids from field-collected *P. gossypii* in Texas. Noyes (2000) suggested that the paratypes might be local contaminations of the laboratory culture in Trinidad. *Anagyrus loecki* was not found among specimens collected in the fields of Texas.

The reported hosts of *A. loecki* are *P. madeirensis*, *Dysmicoccus* nr. *hurdi* McKenzie (host of holotype), and the papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink. The use of *A. loecki* in biological control of these economically important mealybug species has been limited. *Anagyrus loecki* was released, as part of a parasitoid complex, against the papaya mealybug in the Dominican Republic and Puerto Rico. The papaya mealybug biological control program has reported a 97% reduction in the mealybug populations (D. E. Meyerdirk, personal communication).

OVERVIEW OF RESEARCH OBJECTIVES

The biology of *A. loecki* is unknown. Information on the development, reproductive behavior and mealybug-parasitoid interactions are essential to the evaluation of *A. loecki* as a biological control agent of *P. madeirensis*. In my doctoral dissertation research, I examine three important aspects of the biology of *A. loecki*:

1) the interactive effects of temperature, mating status and food supplements on the development, survival and reproduction of *A. loecki*;

2) the preference and suitability of different mealybug developmental stages for *A. loecki*; and
3) the functional and reproductive responses of *A. loecki* to varying host densities.

The effects of temperature, mating status and food supplement on development, survival and reproduction

The life cycle of an insect can be divided into two phases: the immature developmental period between egg deposition and adult eclosion, and the period of adult activities (often referred to as adult longevity) that includes courtship, mating and reproduction. Some species in the temperate zone or those facing periodic adverse environmental conditions may have a period of diapause or dormancy. A comprehensive understanding of the life cycle of an insect should include studies on both the immature and adult stages.

The first step in evaluating a potential biological control agent often involves an understanding of the natural enemy's life history on the target pest. Many biotic and abiotic factors influence the life history and effectiveness of predators and parasitoids. For parasitoids, the biotic factors include host stage or size, host species, superparasitism, competition and plant secondary chemicals (Jervis and Copland 1996). Environmental factors that may influence the life cycle of parasitoids include temperature, humidity and photoperiod (Jervis and Copland 1996). Temperature appears to be the most important environmental factor and influences the developmental rate, longevity, fecundity, reproduction and foraging activities of parasitoids (e.g. Sagarra et al. 2000a, Matadha et al. 2004, Pratissoli et al. 2004) and their hosts (e.g. Cockfield and Potter 1987, Mani 1989, Perez-Mendoza et al. 2004). The basis of IPM is the phenology of the pest and natural enemy populations (Logan 1988). An understanding of the thermal requirements of the pests and their natural enemies is useful in selecting biological control agents

that are best adapted to the environmental conditions experienced by the pests (Jervis and Copland 1996) and in predicting the distribution range of the natural enemies (Kontodimas et al. 2004).

The effect of ambient temperature on the developmental rate of an insect is intrinsically linked to its effect on the metabolic response of the insect (Howe 1967). The enzymatic activities related to arthropod development are proportional to the ambient temperature within a favorable range. As a result, within the favorable temperature range the developmental rate of all stages increases with temperature. Above or below the favorable temperature range, the enzymes are either inactivated or destroyed. Consequently, the development rate slows down as the upper or lower developmental threshold is approached, and may terminate above the developmental thresholds.

Within the medium temperature range of 15 to 30^{o}C (Gilbert et al. 1976) the presumably linear relationship between ambient temperature and developmental rate allows for the calculation of thermal requirements for development. The classical view assumes that the completion of one development stage requires the accumulation of definite amount of heat energy over the period of developmental time (Hodek and Honěk 1996). When expressed mathematically the relationship can be described in a thermal summation equation (Wagner et al. 1984):

$1/D = bT + a$.

D is the developmental time, T is the ambient temperature (in ^{o}C), and *a* and *b* are the regression parameters obtained by fitting the observed data to the linear regression equation. The thermal summation equation is easy to use and allows calculation of two important biological parameters: the lower developmental threshold (t_{min}) and the thermal constant (K). The lower

developmental threshold is the temperature at which the insects cease further development, and is extrapolated by the equation

$t_{min} = - a/b$.

The thermal constant is the number of degree-days (DD) above t_{min} for the completion of a developmental stage, or

$K = 1/b$.

The actual relationship between ambient temperature and developmental rate of insects is not linear because the rates of enzymatic activities increase exponentially, instead of linearly, with temperature (Gilbert et al. 1976). The development of insects often decelerates near developmental thresholds. As a result, the linear relationship could only be applied in the medium temperature range. The thermal summation model could suffer from inaccuracy at extreme temperatures when the developmental rates deviate from the assumed linear relationship. Consequently the linear thermal summation model may overestimate the lower developmental threshold of insects (Fantinou et al. 2003). At the same time, the thermal summation equation fails to estimate the upper developmental threshold. Several non-linear approximations were proposed to better describe the relationship between ambient temperature and insect developmental rate (Wagner et al. 1984). Some of these non-linear models assumed the thermal relationship to be sigmoid or logistic whereas others include empirical or biological parameters in the models to better simulate the responses of insects to the changes in temperature.

Both linear and non-linear models have been used extensively in biological studies of parasitoids and predators. The linear thermal summation model provided excellent fit ($r^2 = 0.99$) to the empirical data on the development rate of *Anagyrus pseudococci* (Girault), a parasitoid of

the vine mealybug, *Planococcus ficus* (Signoret) (Daane et al. 2004). Using the parameters estimated by the linear model, the lower developmental threshold of *A. pseudoccocci* was estimated at 11.6^{o}C and the thermal constant was 223.5 DDs. By fitting the data set to a non-linear model (Wang et al. 1982), Daane et al. (2004) obtained a better fit (r^2 = 0.997) and were able to estimate the upper developmental threshold (36.0^{o}C), the optimum temperature for development (24.7^{o}C) and the exponential rate of increase (0.18). Kontodimas et al. (2004) concluded that the linear thermal summation model and the non-linear Lactin model were the most useful models for modeling the development of the mealybug predators, *Nephus includens* (Kirsh) and *Nephus bisignatus* (Boheman) (Coleoptera: Coccinellidae).

Many encyrtid parasitoids are arrhenotokous, meaning that virgin females produce only male progeny from unfertilized eggs, while the mated females are capable of producing both male and female progeny. Many parasitoids are also capable of regulating progeny sex ratio by producing fertilized and unfertilized eggs in specific sequences (Waage 1986). Thus, the mating status of a female parasitoid has significant impact on its sex allocation pattern. Temperature also influences the reproductive and foraging activities of the parasitoids. The rate of egg production, and thus the fecundity, of parasitoids varies with temperature (van Lenteren et al. 1987, Rosenheim and Rosen 1991). Parasitoids often change their reproductive behavior and sex allocation according to egg load (Rosenheim and Rosen 1991). Because of the influence of temperature on egg load, it is reasonable to assume that there is an indirect relationship between temperature and sex allocation pattern. Few studies have investigated the possible interactive relationship between temperature experienced by and mating status of the parasitoid.

Adult longevity of a parasitoid is affected by the occurrence of host feeding, availability of other carbohydrate- or protein-rich food sources, and body size (Jervis and Copland 1996).

Adult longevity is also tightly linked to the dispersal, foraging and mating activities of an insect. Most adult parasitoids require food, such as host hemolymph, honeydew, nectar, pollen, or other carbohydrate- or protein-rich substitutes, to maintain physiological vigor and obtain energy for locomotion. Adult longevity is often reduced in the absence of such food sources (Jervis et al. 1992). Access to host, nectar or honey sources and low saturation deficit (i.e. high relative humidity) increased the longevity of *C. perminutus* (Davies et al. 2004). Host feeding allowed the parasitoid *Eupelmus vuiletti* (Crawford) (Hymenoptera: Eupelmidae) to increase its egg production and longevity (Giron et al. 2004). The longevity and lifetime fecundity of *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) were significantly increased when the parasitoids were allowed access to alyssum flowers [*Lobularia maritima* (L.), Brassicaceae] (Berndt and Wratten 2005). Honey-fed *A. kamali* survived longer than individuals not supplied with any food source (Sagarra et al. 2000a). Provision of carbohydrate or protein sources, often during shipment of biological control agents, can extend the longevity of adult parasitoids.

Parasitoid adult longevity is also affected by abiotic factors such as temperature, humidity and photoperiod (Jervis and Copland 1996). The phenomenon of reduced adult longevity at higher temperature is well-documented in many parasitoid species. The parasitoid *Encarsia citrina* Craw (Hymenoptera: Aphelinidae) survived seven days longer at 17.5^{o}C than at 27.5^{o}C (Matadha et al. 2004). The longevity of three mealybug parasitoids (*A. pseudococci*, *L. abnormis* and *L. dactylopii*) was significantly reduced when the temperature increased form 18^{o}C to 30^{o}C (Tingle and Copland 1989).

Many biological control agents are shipped in cool storage. The effects of cool storage on the survival of these insects and their effectiveness after release had been studied in some

encyrtid parasitoids. Honey-fed *Tachinaephagus zealandicus* Ashmead (Hymenoptera: Encyrtidae) parasitized 3 times more fly pupae than those that were starved (Ferreira de Almeida et al. 2002b). Few studies have investigated the combined effects of temperature and food supplements on the mortality of the biological control agents and their longevity and efficiency after release. Female *A. kamali* that were supplied with pure honey lived more than 30 days whereas the starved parasitoids died within 2 days (Sagarra et al. 2000a). The longevity of honey-fed *A. kamali* was extended for 10 days at 20^{o}C compared to 27^{o}C. Males and females of *T. zealandicus* survived longer in lower storing temperature and when supplied with honey solution (Ferreira de Almeida et al. 2002a). Neither study analyzed for the interaction between temperature and food sources.

In the first study, I examine the interactive effects of temperature and mating status on the development and reproduction of *A. loecki*. Data collected in the developmental study allows the calculation of thermal requirements for the development of *A. loecki*. I also study the interactive effects of temperature and food provision on the survival and reproduction of *A. loecki*. Results of this study will be useful in planning mass rearing program and in determining the suitable temperature range of the parasitoid.

Host stage preference and suitability

Vinson (1976) defined host selection as the first three steps of parasitoid foraging behavior: host habitat selection, host location and host acceptance. Pak et al. (1986), however, restricted host selection to only the host acceptance phase, i.e. the process by which a parasitoid determines the acceptability of a host when it is within close proximity. This classical model is hierarchical and deterministic, and is concerned with the mechanisms by which a female selects a particular type of host for oviposition (Mackauer et al. 1996). An alternative model of host

selection takes into consideration the physiological states and experience of the parasitoid and the mortality risk of foraging (Godfray 1994). Host preference can be established when the relative frequency of host types parasitized is higher than the relative frequencies of host types available (Hopper and King 1984). Preference in large part is determined by the parasitoid species, host species, and host developmental stage or size.

An understanding of parasitoid foraging behavior in relation to its host stage preference is important to the study of parasitoid life history, host-parasitoid interaction, population dynamics and community structures. A parasitoid foraging in an aggregated host population, such as that of a mealybug population, often encounters hosts of different developmental stages. Due to the differences in body size among the host stages, these hosts represent parcels of resources of different quantities and qualities. Large hosts may contain a high amount of resources for the development of the parasitoid larvae and thus more profitable hosts. At the same time, these large hosts may not be the most suitable hosts for parasitoid development because they are defended behaviorally, morphologically or physiologically. Small hosts, although not well defended and requiring less time to handle, may not contain enough resources to support the complete development of the parasitoid larvae. As a result, host stage often has significant impact on the development, survival and reproduction of the foraging parasitoid.

Host stage selection pattern may be different between idiobiont and koinobiont parasitoids. Idiobiont parasitoids kill or paralyze their hosts immediately after parasitism and do not allow the hosts to continue growth. As a result, each host stage or size encountered by an idiobiont parasitoid represents a fixed amount of resources. Host stage selection is more critical for idiobiont parasitoids because they have to select the most profitable or suitable host stage for the development of their offspring (Godfray 1994). By contrast, koinobiont parasitoids, such as

A. loecki, allow the development of their hosts to resume after parasitism. A koinobiont parasitoid is able to parasitize a wider range of host stages or body sizes, although preference for larger hosts is often demonstrated. Small hosts are comparatively more acceptable to the koinobiont parasitoids than to the idiobiont parasitoids because these small hosts grow into larger individuals thus providing more resources for the development of the koinobiont parasitoid larvae. The size of the hosts at the time of parasitism may be unrelated to the size at the time of mummification (Godfray 1994). There may be tradeoffs between the ability to develop in younger hosts and the developmental time and survival (Godfray 1994).

The foraging behavior of the mealybug parasitoids of Encyrtidae appears to be similar among the different species studied (Boavida et al. 1995, Bokonon-Ganta et al. 1995, Karamaouna and Copland 2000b, Joyce et al. 2001). The parasitoids searched a host habitat by touching the surface with antennae. The sensilla on the antennae functioned to detect the presence of kairomones emitted by the hosts or left on the substrate surface. Once a host was encountered, the first phase of host discrimination begins. The potential host was repeatedly examined for a prolonged duration and may or may not be accepted for oviposition. If the host was accepted for oviposition, the parasitoid turned its body around and inserted its ovipositor by a 'sawing' or 'thrusting' movement. In some species, a 'pumping' movement of the abdomen occurred during egg deposition (Cadée and van Alphen 1997). If the host was neither accepted for oviposition nor recognized when encountered, the parasitoid continued to search for hosts. The parasitoids were also able to discriminate parasitized and unparasitized hosts (Bokonon-Ganta et al. 1995). The searching, examining and ovipositing behaviors were often interrupted by periods of grooming, feeding and resting. The foraging parasitoids could feed on water, honeydew secreted by hosts or host hemolymph exuded from ovipositor insertion wound. Host

feeding on young mealybugs nymphs was observed in *Anagyrus mangicola* Noyes (Bokonon-Ganta et al. 1995), *Gyranusoidea tebygi* Noyes (Boavida et al. 1995), *L. abnormis* (Cadée and van Alphen 1997) and *Leptomastix epona* (Walker) (Karamaouna and Copland 2000b) but not in *Pseudaphycus flavidulus* (Brèthes) (Karamaouna and Copland 2000b).

Most host stage preference studies have two sets of experiments: choice and no-choice tests. Preference for a particular host stage can be determined in the no-choice test by comparing the parasitism rate in the host stages. The relative preference can be demonstrated in the choice tests, as the most preferred host stage is the one suffering from higher parasitism rate compared to other available host stages. Most parasitoids studied to date show a preference for a particular host developmental stage. All host stages were parasitized by *A. mangicola* (Bokonon-Ganta et al. 1995) and *P. flavidulus* (Karamaouna and Copland 2000b). However, the third-instar nymphs of *R. invadens* were preferred over other larval instars and adults whereas the first-instar nymphs were most preferred for host feeding by *A. mangicola* (Bokonon-Ganta et al. 1995). *Pseudaphycus flavidulus* preferred to parasitize larger individuals of *P. viburni* (Karamouna and Copland 2000b). Studies on other parasitic hymenopterans suggested similar preference for particular host stages. When given a choice, *Trybliographa rapae* Westwood (Hymenoptera: Fitigidae), preferred the third-instar larvae of the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae) (Neveu et al. 2000). The aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphididae) parasitized all nymphal instars of *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), but preferred first- and second-instar nymphs of *A. gossypii* and first-instar nymphs of *M. persicae* (Perdikis et al. 2004).

Host stage also has significant impact on the fitness of the parasitoid. Frequently, the most preferred host stage is also the most suitable one. Adults of *P. citri* are the most preferred

hosts of *A. pseudococci* (Islam and Copland 1997). *Anagyrus pseudococci* emerged from the third-instar nymphs and adults of *P. citri* suffered the lowest mortality rate and completed development within the shortest time as compared to those developing in mealybugs of other developmental stages. *Anagyrus kamali* Moursi suffered a high mortality rate (due to egg encapsulation) in its most preferred adult hosts (*M. hirsutus*) (Sagarra and Vincent 1999). However, *A. kamali* developing in adult *M. hirsutus* had the shortest developmental time and the largest progeny body size. *Delia radicum* developing in the third-instar fly larvae completed development more than 10 days earlier and achieved a body size larger than those developing in the first and second instars (Neveu et al. 2000).

A gregarious parasitoid, such as *A. loecki*, has to determine the clutch size (i.e. the number of eggs deposited in a single host) depending on host stage or size. Models regarding parasitoid clutch size have evolved within the frameworks of maximizing fitness either per unit time or per unit host (Godfray 1994). A higher number of progeny could complete development within a single large host than a small host. The gregarious *Anagyrus indicus* Shafee et al. and *P. flavidulus* produced a higher number of progeny per mummy from mealybugs parasitized as adults (Nechols and Kikuchi 1985, Karamouna and Copland 2000a, respectively).

Selection of the most profitable host stage also influences the sex allocation patterns in arrhenotokous parasitoids. A higher proportion of females is often produced from larger hosts. The brood size was larger and the proportion of males was lower when *Metaphycus flavus* (Howard) and *Metaphycus stanleyi* Compere (Hymenoptera: Encyrtidae) were allowed to oviposit in larger hosts (Bernal et al. 1999). Most mealybug parasitoids studied to date showed decreased proportion of males when developing in larger or older hosts (e.g., Nechols and Kikuchi 1985, Boavida et al. 1995, Bokonon-Ganta et al. 1995, Cadee and van Alphen 1997,

Islam and Copland 1997, Sagarra and Vincent 1999, Bertschy et al. 2000, Karamaouna and Copland 2000). Charnov et al. (1981) provided an explanation for the observed sex allocation pattern in relation to host size. Male and female progeny of a parasitoid benefit differently from their development in a larger host. Female progeny developing in larger hosts often achieve a larger adult body size, and consequently a higher fitness. Male progeny also grow larger in large hosts but their body size is not strongly correlated with their mating success (but see van den Aseem 1986). Thus females benefit more than males from being large. A solitary parasitoid, with sex allocation decision governed by the rule proposed by Charnov et al. (1981), will therefore deposit more female eggs in larger hosts and more male eggs in small hosts. In contrast, a gregarious parasitoid could potentially adjust its within-brood progeny sex ratio according to the quality of the host.

In the second study on the ecology of *A. loecki*, I investigate the foraging behavior of the parasitoid in relation to host developmental stage. In addition, I study the impacts of host stage on the development, survival, progeny production and sex allocation patterns in the parasitoid. I will discuss the implications of host stage preference and suitability by a parasitoid in the mass rearing and implementation of biological control program.

Functional and numerical responses

Functional response characterizes the relationship between the rate of attack by a single parasitoid and the host density. Numerical response, on the other hand, describes the change in reproductive output of the natural enemy in relation to the host density. Three types of functional and numerical responses were described: type I, II and III. The ability of a biological control agent to regulate the pest population is dependent upon its functional and numerical responses (Solomon 1949). Functional response analysis is frequently used to predict host-

parasitoid population dynamics. It is also used as a tool in evaluating the effectiveness of the candidate biological control agent and in determining the release rate of the biological control agent.

The practice of biological control rests on the assumption that natural enemies should act in a density-dependent manner (Huffaker et al. 1971). Such natural enemies should cause higher parasitism or mortality among the host or prey populations as the host or prey density increases. There have been controversies over the importance of density-dependent processes in the success of biological control and the host-parasitoid population dynamics (e.g. Stiling 1987, 1989; Brown 1989). What emerged from the debate was a realization of the complexity of host-parasitoid or prey-predator interactions, and their impacts on ecosystem functioning. The numerical and functional responses of parasitoids to host population density depend on the temporal and spatial scales and the life histories of the hosts and parasitoids.

Functional response analyses are in essence an exercise in detecting density dependence. Type I functional response is characterized by the linear increase in the number of hosts parasitized or prey consumed with the increase in host or prey population. When the number of hosts parasitized is converted to the reciprocal proportion of hosts parasitized, the relationship between the proportion of hosts parasitized and the host density becomes constant, suggesting a density-independent relationship. Type II functional response shows an increase in the number of hosts parasitized until a plateau is reached, but an exponential decrease in proportion of hosts parasitized, with host density. Natural enemies exhibiting type II functional response often have an inversely density-dependent relationship with their hosts (Murdoch and Oaten 1975). Type III functional response can be described as a sigmoid-shaped relationship between the number of hosts parasitized and the host density. The proportion of hosts parasitized initially increases, a

characteristic of density dependence, and later drops exponentially after passing a peak. A natural enemy that exhibits type III functional response is expected to produce a more stable population dynamics and be more effective in biological control programs (Hassell et al.1977).

The mathematical representations of the functional response are (Juliano 2001)

Type I: $N_a = aTN_o$,

Type II: $N_a = aTN_o / (1 + aT_hN_o)$, and

Type III: $N_a = (dN_oT + bN_o^2T)/[1- cN_o + dN_oT_h + bN_o^2T_h)$.

In these models, N_a is the number of mealybugs parasitized, N_o is the initial host density, T is the time available for searching and parasitism, *a* is the instantaneous attack rate, the parameters *b*, *c* and *d* are constants related to attack rate, and T_h is the handling time.

Parasitoids frequently demonstrate the type II functional response (e.g. Patel et al. 2003, Lysyk 2004). In contrast, the type III functional response occurred occasionally (e.g. Montoya et al. 2000, Jones et al. 2003). The type I functional response was only observed in a few parasitoid genera (*Eretmocerus* in Jones, et al. 1999, and *Trichogramma* in Mills and Lacan 2004).

The relative rarity of type III functional response may be the result of unnatural experimental protocols in laboratory studies (van Lenteren and Bakker 1978). Under more natural conditions or in laboratory experiments with unrestricted movement, parasitoids would leave the experimental arenas when hosts available for parasitism were depleted. Type II functional response is more common when the parasitoids are confined in an oviposition arena for the entire experimental duration (in a fixed-time experiment) and forced to revisit parasitized hosts. Collins et al. (1981) and Sagarra et al. (2000b) provided empirical data supporting this argument. *Anagyrus kamali* showed a type II functional response when the parasitoid was allowed to forage for 24 hours in a closed oviposition arena (Sagarra et al. 2000b), a result

commonly obtained in experiments with similar design. However, when *A. kamali* was allowed to decide its own residence and foraging time in a variable-time experiment, the resulting functional response was type III.

Numerical response of a parasitoid population is also important to its persistence in the system (Ives and Settle 1996). Despite its importance, numerical response studies have received little attention. Most theoretical models relating parasitoid numerical response to host-parasitoid population dynamics assume a linear relationship between the number of hosts parasitized and the number of progeny produced in the next generation (Hassell 1978). This assumption is true for solitary parasitoids, which eliminate supernumerary larvae. This assumption is not true in the cases of superparasitism and gregarious parasitoids in which multiple parasitoids emerge from each parasitized host (Taylor 1988). The number of parasitoids produced by each gregarious parasitoid or from each superparasitized host is not exact. Competition among parasitoid larvae within a superparasitized host may yield either more or fewer progeny on average than from a singly parasitized host (Taylor 1988). The number of progeny by each gregarious parasitoid is dependent upon the number of eggs deposited per host, which in turn is related to the body size of the host (Taylor 1997).

Density-dependent host mortality is another factor that influences the numerical response of parasitoids (Ives and Settle 1996). Koinobiont parasitoids, which allow the hosts to continue development, are subjected to the same mortality factors experienced by their parasitized hosts. When the mortality of parasitized hosts is dependent on the host density, the mortality risk of parasitoids is also dependent on the host density. For a parasitoid foraging in areas of high host density, the task of finding a suitable host is simplified. However, the mortality risk due to density-dependent host mortality is also increased. This phenomenon produces a trade-off

between parasitoid functional response and numerical response (Ives and Settle 1996). When the host mortality is high, the parasitoid population suffers from high mortality rate and reduces its ability to regulate the host population (May et al. 1981, Ives and Settle 1996). At this point, it is difficult to draw conclusions on the numerical responses of parasitoids to host density because of the small number of experiments and the complications arising due to the confounding factors discussed above.

Few have studied the functional and numerical responses of encyrtid parasitoids of mealybugs. Cloyd and Sadof (2000) conducted an experiment to elucidate the functional response of *L. dactylopii*, which appeared to be type II. However, the parameters of the functional response were not estimated because of the small number of data (R. A. Cloyd, personal communication). *Anagyrus kamali* demonstrated both type II and III functional responses (depending on the experimental setup) when provided with different densities of *M. hirsutus* (2, 5, 10, 25, 50 and 100) (Sagarra et al. 2000b). The number of progeny produced by each *A. kamali* increased with host density up to a threshold where the increase in progeny production rate diminished. Sex ratio of *A. kamali*, on the other hand, did not change with the host density.

The goals of the third experiment are to describe the functional response of *A. loecki*, and study the consequence of host density on the reproduction of the parasitoid. Results from this study will provide important clues to the effectiveness of *A. loecki* against *P. madeirensis* and the sustainability of the *A. loecki*-*P. madeirensis* system.

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Table 1.1 Reported predators and parasitoids of *Phenacoccus gossypii* and *Phenacoccus madeirensis*.

Order/Family	Species	Prey/host species	References	Notes
Diptera				
Syrphidae	*Toxomerus marginata* Macquart	*P. gossypii*	Heming 1936	In New York, possibly *P. madeirensis*; predator species name invalid, maybe *Toxomerus marginatus* (Say).
Coleoptera				
Coccinellidae	*Cryptolaemus montrouzieri* Mulsant	Many	L. S. Osborne, per. com.	Polyphagous species that feed on many mealybug species.
	Diomus austrinus Gordon	*P. madeirensis*	Chong et al. 2005	Also feed on eggs of *Planococcus citri* (Risso).
Neuroptera				
Chrysopidae	*Chrysopa oculata* Say	*P. gossypii*	Heming 1936	In New York, possibly *P. madeirensis*.
	'*Chrysopa*' sp.	*P. gossypii*	Aguilar and Lamas 1980	In Peru, possibly *P. madeirensis*.
	Dichochrysa sp.	*P. madeirensis*	Sinacori and Tsolakis 1994; Miller et al. 2004	
Hemerobiidae	*Sympherobius californicus* Banks	*P. gossypii*	Aguilar and Lamas 1980	In Peru, possibly *P. madeirensis*.
	Sympherobius fallax Navás	*P. madeirensis*	Sinacori and Tsolakis 1994; Miller et al. 2004	

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Neuroptera				
Hemerobiidae	*Sympherobius pygmaeus* (Rambur)	*P. madeirensis*	Sinacori and Tsolakis 1994; Miller et al. 2004	
Hymenoptera				
Aphelinidae	*Coccophagus gurneyi* Compere	*P. gossypii*	Gordh 1979; Peck 1963; Thompson 1953	Potential hyperparasitoid of *L. dactylopii* and *Tetracnemus peregrinus* (Noyes 2003).
Encyrtidae	*Acerophagus coccois* Smith	*P. gossypii*	Ashmead 1900; Van Driesche et al. 1986, 1987; Noyes and Hayat 1994; Noyes 2003	Studies in Columbia may possibly be *P. madeirensis*.
		P. madeirensis	Castillo and Bellotti 1990; Noyes 2003	
			Löhr et al. 1990	As *Phenacoccus grenadensis*.
			Rosen 1969; Beardsley 1976; Van Driesche et al. 1987	Hawaiian and Columbian records are possibly *P. madeirensis*.
	Acerophagus pallidus Timberlake	*P. gossypii*	Flanders 1935; Thompson 1953; Simmonds 1957; Peck 1963; Herting 1972; De Santis 1989; Noyes and Hayat 1994; Noyes 2003	Possibly *P. madeirensis* in some records.
		P. madeirensis	Herting 1972; Noyes and Hayat 1994; Noyes 2003	
	Aenasius flandersi Kerrich (= *phenacocci* Bennett)	*P. gossypii*	Herting 1972; De Santis 1979; Noyes and Hayat 1994; Noyes 2003	

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Hymenoptera				
Encyrtidae	*A. flandersi*	*P. gossypii*	Bennett 1957	As *A. phenacocci*; in Trinidad, possibly *P. madeirensis*.
		P. madeirensis	Noyes 2000	
	Aenasius masii Domenichini	*P. gossypii*	De Santis 1979; Noyes and Hayat 1994; Noyes 2003	Possibly *P. madeirensis*.
			Coquis and Salazar 1976	In Peru, possibly *P. madeirensis*.
	Anagyrus sp.	*P. gossypii*	Herting 1972; Noyes and Hayat 1994; Noyes 2003	
			Salazar 1972	In Peru, possibly *P. madeirensis*.
		P. madeirensis	Löhr et al. 1990	As *P. grenadensis*.
			Boussienguet and Neuenschwander 1989; Neuenschwander et al. 1987; Noyes and Hayat 1994	
	Anagyrus diversicornis (Howard)	*P. gossypii*	Kerrich 1982; Van Driesche et al. 1986, 1987; De Santis 1989	As *Apoanagyrus diversicornis*; possibly *P. madeirensis*.
		P. madeirensis	Noyes 2000	
	Anagyrus elgeri (Kerrich)	*P. madeirensis*	De Santis 1989; Kerrich 1982; Noyes and Hayat 1994; Noyes 2003	As *P. grenadensis*.

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Hymenoptera				
Encyrtidae	*Anagyrus fusviventris* Girault	*P. gossypii*	Viggiani and Battaglia 1983	Laboratory rearing on *P. madeirensis*?
			Noyes and Hayat 1994; Noyes 2000; Noyes 2003	
	Anagyrus loecki Noyes & Menezes	*P. madeirensis*	Noyes 2000; Noyes 2003	
	Anagyrus pseudococci (Girault)	*P. gossypii*	De Santis 1979; Noyes and Hayat 1994; Noyes 2003	Possibly *P. madeirensis*.
	Anagyrus sinope Noyes & Monezes	*P. gossypii*	Noyes 2000; Noyes 2003	
		P. madeirensis	Noyes 2000; Noyes 2003	
	Blepyrus insularis (Cameron)	*P. madeirensis*	Boussienguet and Neuenschwander 1989; Noyes & Hayat 1994; Noyes 2000; Noyes 2003	
	Cheiloneurus carinatus Compere	*P. madeirensis*	Herting 1972; Noyes 2003	As primary or secondary parasitoid.
	Chrysoplatycerus ferrisi Timberlake	*P. gossypii*	Kerrich 1978; Noyes and Hayat 1994; Noyes 2003	
	Coccidoxenoides perminutus Girault	*P. madeirensis*	Herting 1972; Noyes 2003	Laboratory rearing.

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Hymenoptera				
Encyrtidae	*Dicarnosis ripariensis* Kerrich	*P. gossypii*	Kerrich 1978; Noyes and Hayat 1994; Noyes 2003	
	Ericydnus lamasi (Domenichini)	*P. gossypii*	Salazar 1972; De Santis 1979; De Santis 1983; Noyes and Hayat 1994; Noyes 2000; Noyes 2003	Possibly *P. madeirensis*.
	Gryranusoidea sp.	*P. madeirensis*	Boussienguet and Neuenschwander 1989; Noyes and Hayat 1994	
	Gryranusoidea phenacocci (Beardsley)	*P. gossypii*	Beardsley 1969; Noyes and Hayat 1994; Noyes 2003	Specific to *P. gossypii*; record in Hawaii was possibly *P. madeirensis*.
	Holcencyrtus sp.	*P. gossypii*	Salazar 1972; Noyes and Hayat 1994; Noyes 2003	Reported as *Coelaspidia* sp.; host possibly *P. madeirensis*.
	Holcencyrtus myrmicoides (Compere & Zinna)	*P. madeirensis*	Herting 1972; Noyes 2003	Laboratory rearing.
	Leptomastidea sp.	*P. gossypii*	Coquis and Salazar 1976; Noyes and Hayat 1994; Noyes 2003	In Perus, possibly *P. madeirensis*.

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Hymenoptera				
Encyrtidae	*Leptomastidea abnormis* (Girault)	*P. gossypii*	Dozier 1932; Heming 1936; Thompson 1954; Peck 1963; Gordh 1979; Trjapitzin 1989; Noyes and Hayat 1994; Noyes 2000; Noyes 2003	Possibly *P. madeirensis*.
	Leptomastix sp.	*P. madeirensis*	Boussienguet and Neuenschwander 1989; Noyes & Hayat 1994; Noyes 2003	
	Leptomastix dactylopii Howard	*P. gossypii*	Bess 1939	Laboratory rearing.
			Fullaway 1946; Tachikawa 1963	Tachikawa (1963) based on Fullaway (1946); as laboratory rearing from a mixed culture of *P. gossypii* (possibly *P. madeirensis*) and *P. citri*.
			Peck 1963; Gordh 1979; Noyes and Hayat 1994; Noyes 2000; Noyes 2003	Possibly *P. madeirensis*.
		P. madeirensis	Donald 1956; Herting 1972; Prinsloo 1983; Noyes and Hayat 1994; Noyes 2000; Noyes 2003	
	Metanotalia madeirensis (Walker)	*P. madeirensis*	Zuparko 1995	

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Hymenoptera				
Encyrtidae	*Prochiloneurus* sp.	*P. gossypii*	Coquis and Salazar 1976; Noyes and Hayat 1994	Possibly *P. madeirensis*; as primary or facultative hyperparasitoid.
	Prochiloneurus bolivari Mercet	*P. madeirensis*	Boussienguet and Neuenschwander 1989; Noyes & Hayat 1994; Noyes 2003	As primary or hyperparasitioid.
	Prochiloneurus insolitus (Alam)	*P. madeirensis*	Neuenschwander et al. 1987	As hyperparasitoid of *Anagyrus lopezi* (De Santis).
	Prochiloneurus seini (Dozier)	*P. gossypii*	Salazar 1972; Noyes and Hayat 1994	Possibly *P. madeirensis*; as primary or hyperparasitoid.
	Pseudaphycus angelicus (Howard)	*P. gossypii*	Flanders 1935; Thompson 1954; Peck 1963; Herting 1972; Gordh 1979;	Some records could be *P. madeirensis*.
		P. madeirensis	Herting 1972	Laboratory rearing.
	Pseudaphycus mundus Gahan	*P. gossypii*	Gahan 1946; Peck 1963; Herting 1972; Gordh 1979; Noyes and Hayat 1994; Noyes 2003	Laboratory rearing.
	Zarhopalus zancles Noyes	*P. madeirensis*	Noyes 2000; Noyes 2003	Reported only from *P. madeirensis.*
Pteromalidae	*Pachyneuron eros* Girault	*P. gossypii*	De Santis 1979; Noyes 2003	Possibly *P. madeirensis*.

Table 1.1 Continued.

Order/Family	Species	Prey/host species	References	Notes
Hymenoptera				
Signiphoridae	*Chartocerus* sp.	*P. madeirensis*	Noyes 2000	Hyperparasitoid of *A. loecki* in *Dysmicoccus* nr. *hurdi* and *Paracoccus marginatus*; members of the genus are mainly hyperparasitoids.
	Chartocerus dactylopii (Ashmead)	*P. gossypii*	Gordh 1979; Noyes 2003	As predator (?) and hyperparasitoid.
	Chartocerus niger (Ashmead)	*P. gossypii*	Herting 1972, 1977; Noyes 2003	As primary (?) or hyperparasitoid.

CHAPTER 2

INFLUENCE OF TEMPERATURE, MATING STATUS AND FOOD SOURCES ON LIFE HISTORY CHARACTERISTICS OF THE MEALYBUG PARASITOID *ANAGYRUS LOECKI* (HYMENOPTERA: ENCYRTIDAE)[1]

[1] Chong, J.-H., and R. D. Oetting. 2005. To be submitted to Environmental Entomology.

ABSTRACT *Anagyrus loecki* Noyes & Menezes (Hymenoptera: Encyrtidae) is a parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), a common pest in the greenhouses of the southeastern United States. The objectives of this study were to assess the influences of temperature, mating status and food sources on the development, survival, longevity, progeny production, and progeny sex ratio of *A. loecki*. *Anagyrus loecki* is an arrhenotokous parasitoid. Virgin females produce only male progeny whereas mated females produce both male and female offspring with the proportion of males between 0.34 and 0.41. Parasitism rates increased with temperature and averaged from 17 to 40%. The number of progeny produced by mated females within 24 h increased from 8 at 15°C to 11 at 30°C, which were 10 and 50% lower, respectively, than that of virgin females. For females of either mating status, average brood sizes ranged between 3 and 5 progeny per mummy. More than 94% of parasitoids successfully survived to adulthood between 15 and 30°C. No parasitoid completed development at 35°C. The developmental times of *A. loecki* were 55, 25, 17, and 12 d at 15, 20, 25 and 30°C, respectively. The lower developmental threshold and thermal constant of female parasitoids, estimated from the linear thermal summation equation, was 11°C and 227 DD, respectively. Male and female *A. loecki* fed with diluted honey and held at 15°C lived for 32 and 53 d, respectively, which were significantly longer than the individuals fed only with distilled water or starved at higher temperatures. In all temperature/food source combinations, female parasitoids survived longer than males. The lifetime fecundity of mated *A. loecki* averaged 77 progeny. Individuals fed with honey solution produced significantly more progeny than those starved or fed with only distilled water.

KEY WORDS *Anagyrus loecki*, *Phenacoccus madeirensis*, developmental time, reproduction, adult longevity, biological control.

The Madeira mealybug, *Phenacoccus madeirensis* Green, is an important pest in greenhouse ornamental production of the southeastern United States. The Madeira mealybug has a cosmopolitan distribution and a host range of more than 40 plant families (Ben-Dov 1994). Chong et al. (2003) provided life history information of this mealybug species reared at different constant temperatures from 15 to 25°C. A female Madeira mealybug completes development in 30 and 66 d at 25 and 15°C, respectively. After a pre-oviposition period of 1 wk, a female produces up to 600 eggs in 1-2 wks. More than 75% of the eggs eventually resulted in adults. Without sufficient control, a highly fecund Madeira mealybug population has the potential of reaching damaging levels in a relatively short period of time. Successful management of the Madeira mealybug requires repeated applications of insecticides targeting the nymphal instars (Townsend et al. 2000). No commercially available biological control agent is currently recommended for the management of the Madeira mealybug.

Anagyrus loecki Noyes & Menezes is an encyrtid parasitoid of the tribe Anagyrini, whose members are almost exclusively mealybug parasitoids (Noyes and Hayat 1994). *Anagyrus* is a genus comprising about 200 species worldwide (Noyes and Hayat 1994). Many *Anagyrus* species have been used successfully as biological control agents against mealybugs, including *A. mangicola* Noyes against the mango mealybug, *Rastrococcus invadens* Williams (Neuenschwander et al. 1994, Bokonon-Ganta et al. 2002), *A.* (= *Epidinocarsis* or *Apoanagyrus*) *lopezi* (De Santis) against the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero in Africa (Neuenschwander 2001), and *A. kamali* Moursi against the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Sagarra and Peterkin 1999). *Anagyrus loecki* was first described from specimens collected in Costa Rica (Noyes 2000). Its known distribution also

includes Florida, Texas, Mexico, and Saint Kitts Island. Reported hosts of *A. loecki* are the Madeira mealybug, the papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink, and *Dysmicoccus* nr. *hurdi* McKenzie. No biological information was provided with the original description (Noyes 2000). In Florida, *A. loecki* has been identified as a potential biological control agent of the Madeira mealybug (L. S. Osborne, personal communication).

One of the first and most important steps in evaluating the potential of a predator or parasitoid as biological control agent is the study of its life history on the target pest species. Studies on the responses of a parasitoid to various environmental factors provide life history information that is essential for the understanding of the host-parasitoid interactions. Of the myriad of biotic and abiotic factors that may influence the development and effectiveness of biological control agents, temperature appears to be a particularly important factor. Temperature influences the developmental rate, longevity, fecundity, foraging and courtship activities, and the establishment of parasitoids in numerous laboratory and field studies (e.g. Sagarra et al. 2000a, 2000b; Torres et al. 2002; Matadha et al. 2004; Pratissoli et al. 2004; Arai and Mishiro 2004). Higher ambient temperature can increase the foraging activities of the parasitoids, thus leading to a higher proportion of parasitized hosts (Langer et al. 2004). Higher temperature can increase the fecundity of parasitoids (Mani and Krishnamoorthy 1992). On the other hand, increased temperature can reduce adult longevity (Matadha et al. 2004). Developmental rate of immature parasitoids often slows down at extremely high and low temperatures, representing the upper and lower developmental thresholds respectively (Tingle & Copland 1988). Information on the effects of temperature on parasitoid development and survival is crucial to the elucidation of numerical relationships between the parasitoid and its hosts, the design and implementation of

mass-rearing programs, and the prediction of the parasitoid population dynamics and distribution.

Mating status influences reproductive longevity, fecundity and progeny sex ratio of parasitoids (van Lenteren et al. 1987). Many encyrtid parasitoids are arrhenotokous, meaning that virgin females produce only male progeny from unfertilized eggs, while mated females are capable of producing both male and female progeny. Many parasitoids are also capable of regulating progeny sex ratio by producing fertilized and unfertilized eggs in specific sequences (Godfray 1994). For gregarious parasitoids, such as *A. loecki*, clutch size (the number of eggs deposited in a single host) and progeny sex ratio are dependent upon the availability of mature eggs (Rosenheim and Rosen 1991). Since temperature has significant influence on the maturation of eggs (Rosenheim and Rosen 1991) and the courtship and oviposition activities of adult parasitoids (Langer et al. 2004), there are potential interactions between temperature and mating status of the female parasitoids.

Many adult hymenopteran parasitoids consume carbohydrates from various sources, such as honeydew, extra-floral nectaries, and nectar. The carbohydrates consumed are used by adult parasitoids in physiological maintenance and as fuel for foraging or courtship activities. Carbohydrate food sources thus have significant influences on the adult longevity and fecundity (Jervis and Copland 1996). With limited life expectancy under situations of starvation, parasitoids may produce larger clutch sizes than they would otherwise under situations of abundant food sources (Roitberg et al. 1993). It is a common practice to add a food supplement to maintain high survival of parasitoids during shipment.

In this study, we present an examination of the life history characteristics of *A. loecki* in relation to temperature, mating experience, and food treatments. The objectives of this study

were to address the following questions: 1) Do temperature and mating status affect parasitism rate, developmental rate, progeny production, brood size, and sex ratio of *A. loecki*? 2) What is the effect of temperature and availability of different food sources on the longevity of the adult parasitoids? 3) Does lifetime fecundity, brood size and progeny sex ratio differ among females as a result of mating status or feeding treatments? The results of this study will be useful in understanding the host-parasitoid relationship between *A. loecki* and *P. madeirensis* and predicting the potential of *A. loecki* as a biological control agent of *P. madeirensis*.

Materials and Methods

Experimental Conditions. All experiments were conducted in environmental chambers (model I-35VL, Percival Manufacturing Co., Boone, IA) maintained at one of five constant temperatures (15, 20, 25, 30 and 35^{o}C) and relative humidity of 90 ± 3%. A photoperiod of 14 h was maintained with fluorescent lighting within the environmental chambers. The air temperature and relative humidity within the environmental chambers were monitored with portable StowAway temperature and relative humidity loggers (Onset computer Corporation, Pocasset, MA) at 15-min intervals.

Maintenance of Insect Cultures. A colony of Madeira mealybugs was reared on sprouted russet potatoes (*Solanum tuberosum* L.) in an insectary at the University of Georgia, Griffin Campus, Griffin, GA, maintained at a temperature of 25-28^{o}C and a photoperiod of 16:8 L:D. The colony was established with eggs collected from a colony maintained on coleus (*Solenostemon scutellarioides* Thonn.) in greenhouses at the Griffin Campus. To standardize the quality of the mealybug hosts, only adult female mealybugs of body length 2-2.5 mm were used in this study.

The initial culture of *A. loecki* was received from the University of Florida, Mid-Florida Research and Education Center, Apopka, FL in September 2000. This and subsequent reintroductions successfully established viable colonies in greenhouses and laboratories at the Griffin Campus. The colonies were maintained on Madeira mealybugs reared on either coleus in the greenhouses or sprouted russet potatoes in the laboratory. Mummies were collected from the laboratory colonies, isolated individually in gelatin capsules (no. 1, Eli Lilly and Co., Indianapolis, IN), and reared to adult emergence in an environmental chamber maintained at 25^{o}C and 14:10 (L:D) h. To collect virgin female parasitoids for the developmental experiments, mummies were checked every 4 h and females that emerged from single-sex broods were removed and kept in plastic jars (5.5 liter) in the absence of males. Female parasitoids that emerged from the mixed-sex broods were collected along with males for the experiments involving mated females. The female parasitoids were visually graded and only those of the same size (over 1.2 mm in body length) were used in the experiments. Female parasitoids used in the lifetime fecundity and longevity experiments were prepared in a similar procedure within 24 h of emergence.

Influence of temperature and mating status on parasitism, development, and progeny production. This experiment had a two-way factorial design with temperature and mating status as the experimental factors. Mated and virgin females were kept in separate plastic jars containing sprouted russet potatoes infested with 100-200 Madeira mealybugs of various developmental stages to gain oviposition experience for 72 h before the experiment. An excised chrysanthemum (*Dendrathema* x *grandiflora* Kitam.) leaf with the petiole inserted through a hole drilled at the bottom of a Petri dish (100 by 20 mm) and submerged in a cup of water was used as the experimental unit. Each chrysanthemum leaf was infested with 10 adult female

Madeira mealybugs (constituting a mealybug cohort) collected from the insectary colony. Each mealybug cohort was exposed to a mated or virgin female parasitoid for 24 h in an environmental chamber maintained at one of the five constant temperatures. Each Petri dish was covered with chiffon to allow ventilation. After removal of the parasitoid, the mealybugs were returned to the environmental chamber and incubated until the formation of mummies. A total of 50 replicates was prepared for each temperature/mating status combination at 15-30^{o}C, and 75 replicates at 35^{o}C. The mealybug cohorts were examined 7 (at 25, 30 and 35^{o}C) or 14 d (at 15 and 20^{o}C) after exposure to the parasitoids. The mummies were collected, isolated in individual gelatin capsules, and incubated in designated environmental chambers until adult emergence. The emerged parasitoids were counted and sexed. All mummies collected in the experiment were dissected and any dead adult or immature parasitoids within the mummies were counted. The duration of development was also determined for individual parasitoid.

Parasitism rate, progeny emergence rate, and the number and sex ratio of progenies were determined for each mated or virgin reproductive female parasitoid. The parasitism rate was calculated as the number of mummies collected divided by the total number of mealybugs in a cohort. The number of dead parasitoids was used to calculate progeny emergence rate by dividing the number of emerged adults with the sum of live and dead progeny produced by each reproductive female. We made a distinction between the daily fecundity and brood size of each reproductive female parasitoid: the per capita daily fecundity is the number of progeny produced by each reproductive female within 24 h, and the brood size is the number of progeny that emerged from individual mummies. The effects of temperature and mating status on parasitism rate, progeny emergence rate, progeny production, brood size, sex ratio, and developmental time of *A. loecki* were analyzed with two-way Analysis of Variance (ANOVA, PROC GLM; SAS

Institute 1999). When significant difference was detected in any of the above parameters measured, a Tukey's Honestly Significant Difference (HSD) test was used to separate the means.

Several regression models have been proposed to elucidate the relationship between developmental rate of an insect and the ambient temperature (Wagner et al. 1984). One of the most commonly used models is the linear approximation or thermal summation model (Uvarov, 1931; Wagner et al., 1984), which describes the relationship in a linear regression model:

$$1/D = bT + a, \qquad [1]$$

where D is the developmental time, T is the ambient temperature (in ^{o}C), and *a* and *b* are the regression parameters obtained by fitting the observed data to the equation with linear regression analysis (PROC REG; SAS Institute 1999). The linear approximation equation is suitable for extrapolating the relationship between developmental rate and ambient temperature within a moderate range of temperatures, such as 15 to 30^{o}C (Gilbert et al., 1976). The lower developmental threshold ($t_{min} = - a/b$) is the temperature at which the insects cease further development, and the thermal constant ($K = 1/b$) is the number of degree-days (DD) above t_{min} for the completion of a developmental stage.

Influence of temperature and feeding treatment on adult longevity. Male and female adult *A. loecki* were subjected to five constant temperatures (15, 20, 25, 30 and 35^{o}C) and three feeding treatments (starvation, distilled water and 50% honey solution) arranged in a two-way factorial design. Adult parasitoids were collected upon emergence and individually released into a 1-dram glass vial, which was stopped with a cotton ball to prevent the escape of parasitoids and allow ventilation. Food solution was supplied on pieces of filter paper on the bottom of each glass vial. Parasitoids assigned to the starvation treatment received no food solution in the glass vials. The parasitoids were kept at one of the five prescribed temperatures until death. The glass

vials were changed every week to prevent growth of mold. Immediately after the death of the parasitoids, their left hind tibial lengths were measured using an ocular micrometer in a dissecting microscope. Body length is often correlated to adult longevity and fecundity of adult parasitoids (Jervis and Copland 1996). To correlate body length and tibial length, five individuals were randomly selected from each temperature/feeding treatment combination, and their body length and hind tibial length were measured after they died. In this experiment, 30 females and 15 males were prepared for each temperature/feeding treatment combination. Individuals that drowned in excess food solution were excluded from statistical analyses.

The longevity of *A. loecki* is the time elapsed between emergence and death of the adult parasitoids. The effects of temperature and feeding treatment on adult longevity of *A. loecki* were analyzed with two-way ANOVA and the means separated by Tukey's HSD test. To assess the potential of hind tibial length as a meaningful predictor of body length, the body lengths of five individuals from each temperature/feeding treatment combination were first regressed against their tibial lengths (PROC REG). Individual adult longevity was then regressed against hind tibial length to test for the hypothesis of increased longevity with increased body size (PROC REG).

Influence of mating status on lifetime fecundity and progeny sex ratio. Upon emergence, female parasitoids were collected from the gelatin capsules and anesthetized with carbon dioxide. Their hind tibial length were measured and only female parasitoids with length of 0.40 mm were selected for this experiment to minimize the difference in body size among the females, and the effect of body size on fecundity and longevity. Selected females were subjected to one of the three mating status treatments: virgin, exposed to male for 24 h, and continuous male presence. Virgin females were released into the cages without males. Each female

subjected to 'exposed to male for 24 h' treatment was maintained with a single male for 24 h immediately after adult emergence. The remaining females were caged in continuous presence of an equal number of males. Selected females were released individually into cages built of plastic containers (15 by 15 by 15 cm). Holes were cut in the container lids and covered with fine-mesh chiffon to allow ventilation. Each cage contained a sprouted russet potato infested with over 100 mealybugs of various developmental stages. Each treatment was replicated 12 times. The parasitoids and mealybugs were incubated at 25^{o}C and examined daily for the mortality of parasitoids. The parasitoids were moved to new cages containing fresh mealybugs after 14 d in the original cages. Cages were examined daily after the 14th day for the emergence of adult parasitoids until no more parasitoids were recovered from the cages. The progeny were collected, counted and sexed. The effect of mating status on lifetime fecundity, reproductive longevity, and progeny sex ratio of female *A. loecki* was analyzed using a one-way ANOVA (PROC GLM). Means were separated with Tukey's HSD test.

Influence of feeding treatment on lifetime fecundity, brood size and progeny sex ratio. Thirty six females with hind tibial length of 0.40 mm (determined as described in previous paragraph) were each paired with one adult male upon emergence and randomly assigned to one of three feeding treatments (starvation, distilled water, and 50% honey solution). Each female was released into a Petri dish (60 by 15 mm) supplied with a piece of filter paper wetted by the designated food solution. Females subjected to the starvation treatment received only dry filter paper. Ten Madeira mealybugs in each Petri dish were exposed to the parasitoids for 24 h after which the mealybugs were moved onto an excised chrysanthemum leaf and incubated at 25^{o}C until mummification. The parasitoids were then moved into a new Petri dish containing fresh mealybugs. This process continued until the death of the parasitoids. The

mummies were collected after 7 d, individually isolated in gelatin capsules and incubated at 25°C until adult emergence. The emerged parasitoids were counted and sexed. ANOVA was used to assess the effects of feeding treatment on the lifetime fecundity, reproductive longevity, brood size, and progeny sex ratio of *A. loecki* (PROC GLM). When a significant difference among the means was detected, the means were separated by Tukey's HSD test.

Results

Influence of temperature and mating status on parasitism, development and progeny production. Both temperature and mating status significantly affected the daily parasitism rate of *A. loecki* (Table 2.1). No significant interaction between temperature and mating status on parasitism rate was detected. Between 15 and 30°C, the average parasitism rates ranged between 17 and 40 %. Females of both mating status parasitized more mealybugs as temperature increased. Virgin females consistently parasitized more mealybugs than mated females between 20 and 30°C. Mean parasitism rate of mated females increased from 17 % at 15 °C to 33 % at 30°C. Parasitism rate of virgin females increased from 18 to 40% within the same temperature range. Few mummies were collected at 35°C. Only one replicate, out of a total of 75, yielded a parasitism rate of 40% in the 35°C/virgin combination.

Between 15 and 30°C, progeny emergence rates ranged between 94 and 98% and were not significantly different among the temperatures (Table 2.1). Emergence success was greatest at 30°C for both males and females, with more than 97% of the potential progeny emerged from the mummies. No adult emergence was observed at 35°C. Progeny emergence rates were not different between the mating status treatments at any temperature. Dissection of the mummies did not reveal encapsulation of immature parasitoids by the mealybugs.

The average total number of progeny produced by virgin or mated females within a 24-h period ranged from 8 to 24 (Table 2.2). Both mated and virgin females produced more offspring at higher temperatures especially at 30^{o}C (mean = 23.6 for virgin females and 11 for mated females). Temperature did not have a significant effect on the number of female progeny produced: a mated female produced on average 5.6 female offspring at any temperature. Both temperature and mating status, however, had significant effects on the total progeny number and the number of males. The interaction term between temperature and mating status was also significant. Within a 24-h period, virgin females produced a higher number of progeny than mated females. The numbers of male progeny by mated females were similar at all temperatures. The highest number of male progeny recorded for a single virgin female was 46.

The average brood sizes produced by individual females suggested similar trends as shown in the per capita progeny production within 24 h. On average, 3-5 parasitoids emerged from each mummy, depending on the temperature of incubation and the mating status of reproductive females (Table 2.3). Temperature significantly influenced the number of female progeny within a brood. Mated females ovipositing at 15 and 30^{o}C produced more female offspring than females at 20 and 25^{o}C, although the numbers of male per brood did not differ significantly among the temperatures. On average, 2.5 females and 1 male emerged from each mummy at 15 and 30^{o}C, compared to 2 females and 1 male from mummies at 20 and 25^{o}C. The total number of progeny was significantly influenced by temperature and mating status experienced by the female parasitoids. Significant interaction between the two main factors was also detected. Between 20 and 30^{o}C, virgin females produced on average 0.5 to 1.5 more offspring per brood than the mated females within a 24-h period.

Anagyrus loecki is arrhenotokous. Virgin females produced 100% male broods at all temperatures (Tables 2.2 and 2.3). Mated females produced broods with a proportion of males of 0.3-0.4, with the lowest proportion reported for broods at 15°C. For the mated females, progeny sex ratios calculated from the number of progeny produced per female and the individual brood size were identical.

Male and female *A. loecki* emerged within 24 h of each other, regardless of the mating status of the reproductive females (Fig. 2.1). All individuals within a temperature treatment emerged within 48 h of the first emergence, resulting in very small standard errors for the means of developmental time. The developmental time of both males and females decreased from 55 d at 15°C to only half of that at 25°C, and to 11-12 d at 30°C.

The linear approximation model provided an excellent description for the relationship between temperature and development rate of *A. loecki*, with coefficients of correlation (r^2) of 0.9738 and 0.9739 for females and males, respectively (Fig. 2.2). For females, the equation was

$1/D = 0.0044T - 0.0482$ ($F = 0.2943.6$, $p < 0.0001$; Fig. 2.2A) [2]

Since there was no significant difference between males from the two mating status treatments, the data were pooled before linear regression. Developmental rate of males was best described as

$1/D = 0.0046T - 0.0520$ ($F = 79166.9$, $p < 0.0001$; Fig. 2.2B) [3]

The lower developmental thresholds (t_{min}) were similar between males and females, at 11.3 and 11.0°C, respectively. The thermal constant (K) of females (227.3 DD) was slightly higher than that of male (217.4 DD).

Influence of temperature and feeding treatment on adult longevity. Temperatures, feeding treatments and their interactions significantly influenced adult longevity of both male

and female *A. loecki* (Table 2.4). The highest average longevity for females was 53 d when fed with 50% honey solution at 15°C, and the lowest was 1 d when starved or provided with distilled water at 35°C. Similarly, males survived the longest at 15 °C when fed with honey solution (mean = 32 d) and shortest at 35°C when starved or provided with only distilled water (mean = 1 d). Males had similar or shorter longevity than females in all temperature/feeding treatment combinations. Extremely high temperature (35°C) appeared to be detrimental to the survival of adult *A. loecki*. None of the females and males subjected to 35°C treatment survived more than 7 and 4 d, respectively, regardless of the availability of food solution. Within each temperature treatment, males and females survived considerably longer when fed with 50% honey solution than those starved or provided with only drops of distilled water. Females fed with honey solution lived on average 53 d at 15°C, more than 13 and 7 times longer than starved females and females provided with only distilled water, respectively, at the same temperature. Distilled water did not appear to provide any nutritional value to the physical maintenance and survival. Parasitoids fed with distilled water lived slightly, although not significantly, longer than the starved individuals at all temperatures. Parasitoids fed with the same food survived longer at lower holding temperatures than at higher temperatures. Average longevity of females fed with honey solution was reduced from 53 d at 15°C to 7 d at 35°C. A similar trend was observed in male longevity where the average longevity was reduced to 4 d at 35°C from a maximum of 32 d at 15°C when fed with honey.

Female body length ranged from 0.8 to 1.45 mm. The males were smaller (body length 0.7-1.1 mm). Hind tibial length was a sufficient predictor of female body length, accounting for 70% of the variation (Fig. 2.3A). Hind tibial length of males, however, explained only 36% of variation in the observed data (Fig. 2.3B). Since there were significant interactions of

temperature and feeding treatment on adult longevity, the possible influence of body size (using hind tibial length as a surrogate) on longevity was examined separately for each temperature and feeding treatment combinations. Hind tibial length did not account for more than 50% of the variation in most temperature/feeding treatment combinations, except in males fed with only distilled water at 15^{o}C and honey solution at 25^{o}C (Table 2.5).

Influence of mating status on lifetime fecundity and progeny sex ratio. Virgin female parasitoids lived significantly shorter than females exposed to male for 24 h or females with continuous male presence (Table 2.6). On average, virgin females lived for 13 d at 25^{o}C with excess supply of hosts, while mated females lived 3 d longer. The maximum longevity was 19 d for a mated female, and 16 d for a virgin female. Reproductive periods of the females also differed among the mating status treatments: virgin females were actively ovipositing for only slightly more than half the duration of mated females. Mated females were reproductive as long as they lived. Mated females of either male presence treatments produced similar means of female (53) and male (25) progeny. The total lifetime fecundity of virgin females was significantly lower than that of the mated females (Table 2.6). The daily fecundities of all females were similar over the course of reproduction at an average of 5 progeny per female per day (Fig. 2.4A).

Mating during the first 24 h following emergence ensured sufficient sperm load for the females as suggested by the similar proportions of male progeny between the females mated for only 24 h and the females with continuous male presence (Table 2.6). Progeny sex ratio did not appear to change from one day of oviposition to the next among females of different mating status, although females mated for only 24 h produced lower proportion of male progeny in the later part of progeny emergence period (Fig. 2.4B).

Influence of feeding treatments on lifetime fecundity, clutch size and progeny sex ratio. The starved females lived for less than 3 d and parasitized hosts only on the first 2 d after emergence (Table 2.7, Fig. 2.5A). Females provided with distilled water lived and reproduced for slightly longer than starved females. The longevity and reproductive period were 5 times longer when the females were supplied with fresh 50% honey solution with each transfer to a new host cohort. Because of this reduction of life expectancy due to lack of food sources, many starved females failed to parasitized any mealybug. On average, only 2 mealybugs were parasitized by the starved females over the reproductive period, compared to 4 and 15 by the females provided with distilled water and the females supplied with honey solution, respectively (ANOVA: Feeding treatment, $F = 26.81$, $p < 0.0001$). An individual female fed with honey solution parasitized on average 1-4 mealybugs per day, which was 2-6 times as many mealybugs as starved females and females fed only distilled water within the same day of exposure.

Differences in the parasitism efficiencies among females subjected to the three feeding treatments resulted in significant differences in the number of progeny produced. The number of progeny produced by the females fed with honey solution was about 7 times higher than that by the starved females. The number of mealybugs parasitized and the total number of progeny produced by individual females subjected to any feeding treatment was highest on the first day of exposure to hosts and decreased over the course of reproduction (Fig. 2.5A). Brood sizes were similar among females subjected to different feeding treatments but appeared to be highest on the second day of exposure (Fig. 2.5B). However, the sex ratios were not significantly different among the feeding treatments over the course of the experiment, with about 1 male for every 2 females produced (Table 2.7, Fig. 2.5C).

Discussion

The relationship between temperature and developmental rate of *Anagyrus* spp. has been examined in several studies (e.g., Tingle and Copland 1988, Mani and Krishnamoorthy 1992, Daane et al. 2004). In this study, *A. loecki* developed and survived in the temperature range of 15 to 30°C. This result suggested that *A. loecki* is capable of foraging and developing in greenhouses maintained within the preferred temperature range of its target host (Chong et al. 2003, 2004). The developmental rate of *A. loecki* increased with temperature from 15 to 30°C, similar to the results reported in recent studies on other encyrtid parasitoids (Ferreira de Almeida et al. 2002a, Daane et al. 2004). In some studies, the parasitoids successfully completed development at either lower or higher temperature than this range (Avidov et al. 1967, Daane et al. 2004).

Experimental data indicated that 35°C exceeded the upper thermal threshold for immature development. At this temperature, only 4 mummies were collected from a total of 750 mealybugs exposed to the parasitoids and no mummies yielded any adults. There are two possible reasons for this failure. First, extremely high temperatures may interfere with egg maturation or deposition of *A. loecki*. *Anagyrus pseudococci* (Girault) deposited eggs from 14 to 34°C (Tingle and Copland 1989, Danne et al. 2004). At 35°C, *Anagyrus dactylopii* (Howard) and *A. diversicornis* (Howard) successfully oviposited and developed on *M. hirsutus* and *Phenacoccus herreni* Cox & Williams, respectively (Mani and Krishnamoorthy 1992, Herrera et al. 1989, respectively). These studies suggested that oviposition occurs at temperatures higher than 30°C in some species of *Anagyrus*. Alternatively, ambient temperature above 30°C may represent a true developmental threshold for the immatures. The fact that 4 mummies were collected from the samples suggested that the upper developmental threshold for pupae may be

lower than 35°C but higher for larvae. We did not perform oviposition behavior studies or dissections at 35°C, so we cannot suggest a specific cause for poor performance of *A. loecki* at this temperature.

In the field, insect growth varies depending on the fluctuating temperature experienced by the insects. Conclusions drawn from studies of arthropod development at constant temperatures should be viewed with caution (Liu et al. 1995). Parasitoids may be able to develop in a high temperature that is lethal in the constant temperature experiments but tolerable in the fluctuating temperature experiments. Although three mealybug parasitoids [*A. pseudococci*, *Leptomastix dactylopii* Howard and *Leptomastidea abnormis* (Girault)] did not complete development at 40°C, the species were successfully reared in a temperature regime fluctuating between 26 and 40°C (Tingle and Copland 1988). The linear thermal summation model may overestimate the lower developmental threshold of insects (Fantinou et al. 2003).

Many models have been proposed for the relationship between ambient temperature and developmental rate (Wagner et al. 1984). A linear approximation model can suffer from inaccuracy at extreme temperatures when the developmental rates often deviate from the assumed linear relationship. We chose a linear model in this study because of its ease in use, requirement of limited data, and ability to estimate the lower developmental threshold and thermal constant. The estimated lower developmental threshold for female *A. loecki* was 11.3°C and the thermal constant was 227.3 DD. Although the lower developmental thresholds and the thermal constants were not always estimated in studies on the development of other mealybug parasitoids of the genus *Anagyrus*, the parameters can be easily estimated using the published data. The lower developmental thresholds and thermal constants of females were estimated at 8.6°C and 362.3 DD for *A. diversicornis* (Herrera et al. 1989), 10°C and 282.5 DD for *A.*

dactylopii (Mani and Krishnamoorthy 1992), 11.6°C and 223.5 DD for *A. pseudococci* reared on *Planococcus ficus* (Signoret), 12.3°C and 213.2 DD for *A. pseudococci* attacking *Planococcus citri* (Risso) (Avidov et al. 1967), 13°C and 194 DD for *A. pseudococci* on *P. citri* (Tingle and Copland 1988), and 13°C and 234.2 DD for *A. subalbipes* Ishii on *Pseudococus cryptus* Hempel (Arai and Mishiro 2004), respectively. *Anagyrus loecki* appeared to have a lower developmental thresholds and thermal constants consistent with these species. Determination of the lower developmental threshold and thermal constant is useful in predicting the distribution of a biological control agent. Biological control agents can only establish in a region where the minimal temperature does not fall below the lower developmental threshold and the climatic conditions allow sufficient accumulation of the thermal units (degree-days) until the thermal constant for complete development is reached.

The development of endoparasitoids such as *A. loecki* is intimately linked to the temperature-dependent development of their hosts (Hentz et al. 1998). Thus it is crucial to compare developmental thresholds of the endoparasitoids with those of their hosts. In the laboratory, the Madeira mealybug developed and reproduced on excised leaves within the temperature range of 15-25°C (Chong et al. 2003), although development and oviposition was also successful at 30°C when whole plants were used (Chong et al. 2004). Using data presented in Chong et al. (2003), we estimated that the lower developmental threshold and thermal constant of female Madeira mealybugs are 7.3°C and 540.5 DD, respectively. The lower developmental threshold of the Madeira mealybug was lower and the thermal constant was higher than those of *A. loecki* in this study. The comparison suggested that the host could develop and establish at lower temperature, and take longer to complete development than its parasitoid between 15 and 30°C, a phenomenon observed by Campbell et al. (1974). The life history characteristics of the

Madeira mealybug may be beneficial to the establishment of *A. loecki*. Establishment at lower temperatures may provide a refuge for the mealybugs and ensure the availability of hosts when the parasitoids emerge or are introduced later in the season. Longer developmental times of the mealybugs could ensure prolonged presence of suitable hosts for the parasitoids during the season.

Mating affects longevity, lifetime fecundity and progeny sex ratio of parasitoids (Sagarra et al. 2002). Jervis and Copland (1996) suggested that mating status or mating frequency does not affect the fecundity of arrhenotokous parasitoids since virgin females can lay viable eggs. However, Sagarra et al. (2002) concluded that mated *A. kamali* females have a lower tendency to superparasitize their hosts, thus mated females may parasitize more hosts (8 hosts/d versus 5 hosts/d) and produce more progeny (20 per female compared with 12 per female) than the virgin females. A similar result was also reported for *A. pseudococci* (Avidov et al. 1967). Our results suggest that mating status influenced the daily fecundity of females, but disagree with the conclusions of Sagarra et al. (2002) and Avidov et al. (1967). The interactive effect of mating status and temperature significantly influenced the total number of progeny produced per female and the brood size in this study. Parasitism rate and total number of progeny produced within 24 h of exposure, and brood size of *A. loecki* increased incrementally with temperature between 15 and 30 ^{o}C. Virgin females parasitized significantly more mealybugs, produced more offspring within 24 h, and larger broods than the mated females at every temperature. However, virgin females produced significantly fewer offspring over the reproductive period than females with 24-h or continuous male presence.

Most studies on parasitoids do not investigate the interaction between temperature and the mating status of the reproductive females. Often only the effect of temperature on the

parasitism rate of parasitoids is investigated. Generally, the number or the proportion of hosts parasitized increases with increasing temperature up to a threshold where the parasitism rate declines. The egg parasitoid *Trichogramma ostrinae* Pang & Chen (Hymenoptera: Trichogrammatidae) parasitized increasingly more host eggs from 17-24 ^{o}C, then parasitism rate declined gradually from 28 to 33 ^{o}C (Wang et al. 2004). The percent parasitism of another egg parasitoid, *Telenomus cyamophylax* Polaszek (Hymenoptera: Scelionidae), also increased from 47% at 15 ^{o}C to 97% at 25 ^{o}C and dropped slightly to 82% at 30 ^{o}C, with the highest progeny production at 25 ^{o}C (Foerster and Butnariu 2004). We observed an abrupt termination of parasitism and/or development at 35 ^{o}C, instead of a gradual drop in parasitism rate or number of progeny at higher temperatures.

Courtship and oviposition activities are energy-demanding activities that could divert energy from maintenance and egg production of the parasitoids. However, the lifetime fecundity of virgin *A. loecki* was lower than that of mated females. A mated female produced an average of 78 eggs over her lifetime, similar to that reported for *A. lopezi* (Odebiyi and Bokonon-Ganta 1986) and *A. kamali* (Sagarra et al. 2000a). However, the lifetime fecundity of *Anagyrus* species ranged widely from 14.5 progeny produced by *A. pseudococci* (Avidov et al. 1967) to 600 eggs per female by *A. lopezi* (Fabres 1981, cited by Odebiyi and Bokonon-Ganta 1986).

Progeny sex ratio could be influenced by sperm availability and egg load. Exposure to male for only 24 h was enough to ensure fertilization of all eggs oviposited by a female through her lifetime as evidenced by the similar sex ratio and lifetime fecundity of females of the two male presence treatments. The longevity of mated or virgin females was shorter when they were allowed to parasitize hosts than when the hosts were not available. This reduction in longevity

was likely due to the increased level of activities associated with foraging and oviposition when parasitism was allowed (van den Assem 1996).

Most adult parasitoids require food, such as host hemolymph, host honeydew, nectar, pollen, or other carbohydrate- or protein-rich substitutes, to maintain physiological vigor and obtain energy. Adult longevity often declines in the absence of food sources (Jervis et al. 1992). Both sexes of *A. loecki* lived significantly longer when supplied with honey solution than those provided with distilled water or starved. The females lived longer than the males was possibly due to their larger sizes and energy reserves. There was also a significant interactive effect of temperature and feeding treatment on the longevity of adult *A. loecki*. Adult *A. loecki* fed with the same food survived longer at lower temperatures than higher temperature. A similar phenomenon was observed in *A. kamali* where the females fed with honey at 20 ^{o}C lived significantly longer than the females not supplied with food at the same temperature (40 and 2 d, respectively) and the females fed with honey at 27 ^{o}C (40 and 29 d, respectively) (Sagarra et al. 2000a). Most studies on *Anagyrus* species examined the influence of either various constant temperatures when the parasitoids were fed with honey or different food sources at a single constant temperature. Few studies have been conducted to investigate the interactions between temperature and availability of food in determining longevity of adults. Parasitoids are often released in situations with varying temperature and food availability. In addition, cold storage and food supplementation are common practices when shipping the parasitoids. Therefore, the interactions between temperature and food sources on the longevity of the parasitoids merit more extensive investigations.

Besides the availability of host hemolymph or liquid carbohydrate sources, an additional factor that influences adult longevity is the body size of the parasitoids, measured through

extrapolation from the length of body parts such as body, head or tibia (Jervis and Copland 1996). Body size, measured by hind tibial length, had been shown to be an excellent indicator of the fitness of *A. kamali*: the larger females lived and reproduced for a longer duration, and produced more female offspring per day and over the lifetime than the smaller females (Sagarra et al. 2001). However, the body size was not strongly correlated with the longevity in the adults of *A. loecki* in this study. *Anagyrus loecki* of various body sizes appeared to survive equally well within each temperature/feeding treatment combination. Recently, similar findings were reported for *Celatoria compressa* (Diptera: Tachinidae) where body size of the parasitoid was not correlated to its longevity and fecundity (Zhang et al. 2004).

Adult parasitoids often produce more progeny when fed with more nutritious food sources, such as host hemolymph and liquid sugar sources, compared to those without any food supplements (Jervis et al. 1992). Compared to females deprived of all food sources, honey-fed females lived and reproduced about 5 times longer, and produced 7 times more progeny. For the first 4 d of reproduction, females subjected to different feeding treatments produced similar brood sizes. Effects of food sources on the fecundity of *Anagyrus* species were not investigated in other studies. Ferreira de Almeida et al. (2002b) reported that when fed with honey solution the encyrid parasitoid, *Tachnaephagus zealandicus* Ashmead, killed 3 times more muscoid fly hosts and produced 4 times more progeny per female than when no food was provided. Female parasitoids provided with honey solution lived longer and more active in searching, thus allowing them to parasitize more hosts and produce more progeny.

We can now address the questions presented in this study. Temperature was an important abiotic factor that significantly affected the development, parasitism, progeny production, and adult longevity of *A. loecki*. The availability of carbohydrate sources increased the longevity and

lifetime fecundity of the adult females. Temperature, food availability and mating status of the adult female parasitoids often interact to create a more dynamic and variable response of the parasitoids in relation to their reproductive biology. Results of this study suggested that the reproductive biology of parasitoids is dynamic and dependent upon both environmental factors (such as temperature) and physiological state of the parasitoids (such as sperm load and nutrition). Considering the myriad of biotic and abiotic factors any parasitoid has to face in the field, it is crucial to study the responses in development, survivorship and reproduction of the parasitoid in an interactive manner.

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Table 2.1 Parasitism rate and progeny emergence rate (%, mean ± SEM) per mated and virgin *Anagyrus loecki* females for hosts parasitized during a 24-h period at constant temperatures.

Temperature	Parasitism rate per female		Progeny emergence rate	
°C	Mated	Virgin	Mated	Virgin
15	17.4 ± 2.4bA	18.7 ± 3.8bA	96.4 ± 3.6a	95.7 ± 4.3a
20	25.4 ± 2.4abB	28.8 ± 3.2abA	94.3 ± 2.5a	94.9 ± 2.2a
25	27.8 ± 3.8abB	32.1 ± 3.5abA	95.1 ± 3.3a	99.1 ± 0.6a
30	33.2 ± 4.1aB	40.1 ± 5.9aA	98.0 ± 4.0a	97.4 ± 1.6a
35	0cB	0.8[a]cA	-	0 [a]b
		ANOVA *F* values		
Temperature (Temp)	30.87 ***		6.73 ***	
Mating status (Mate)	3.97 *		1.72 NS	
Temp * Mate	0.33 NS		0.69 NS	

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA, $\alpha = 0.05$).
Mean parasitism or progeny emergence rates followed by the same small letter within each mating status are not significantly different among temperature treatments. Mean parasitism or progeny emergence rates followed by the same capital letter within each temperature treatment are not significantly different between the mating status. (Tukey's HSD, $\alpha = 0.05$).
[a] Mummies collected in only one replicate.

Table 2.2 Number and sex ratio of progenies (mean ± SEM) produced by mated and virgin *Anagyrus loecki* females during a 24-h period at constant temperatures.

Mating status	Temperature °C	Number of progenies			Sex ratio (Proportion of males)
		Female	Male	Total	
Mated	15	5.8 ± 0.8	2.3 ± 0.4aB	8.1 ± 1.1aA	0.29 ± 0.04bB
	20	5.4 ± 0.6	3.1 ± 0.5aB	8.5 ± 0.8aB	0.41 ± 0.03aB
	25	5.4 ± 0.8	2.9 ± 0.4aB	8.1 ± 1.1aB	0.38 ± 0.04aB
	30	7.6 ± 1.0	3.4 ± 0.4aB	11.0 ± 1.4aB	0.35 ± 0.03aB
Virgin	15	0	9.3 ± 1.3bA	9.3 ± 1.3bA	1aA
	20	0	11.1 ± 1.2bA	11.1 ± 1.2bA	1aA
	25	0	13.7 ± 1.7bA	13.7 ± 1.7bA	1aA
	30	0	23.6 ± 1.7aA	23.6 ± 1.7aA	1aA
			ANOVA *F* values		
Temperature (Temp)		1.24 NS[a]	6.69 **	7.52 ***	13.28 ***
Mating status (Mate)		-	139.88 ***	25.29 ***	2693.89 ***
Temp x Mate		-	9.08 **	5.28 **	0.44 NS

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA, $\alpha = 0.05$).

Mean progeny numbers or sex ratio followed by the same small letter are not significantly different among temperature treatments within a mating status. Mean progeny numbers or sex ratio followed by the same capital letter are not significantly different between mating status within a specific temperature (Tukey's HSD, $\alpha = 0.05$).

a Only temperature effect on female progeny production by mated reproductive female was analyzed by one-way ANOVA.

Table 2.3 Brood size and progeny sex ratio (mean ± SEM) produced by mated and virgin *Anagyrus loecki* females during a 24-h period at constant temperatures.

Mating status	Temperature °C	Number of progenies			Sex ratio (Proportion of males)
		Female	Male	Total	
Mated	15	2.7 ± 0.2a	1.1 ± 0.1aB	3.9 ± 0.2aA	0.29 ± 0.04bB
	20	1.9 ± 0.1b	1.0 ± 0.1aB	2.8 ± 0.2bB	0.40 ± 0.04aB
	25	2.0 ± 0.1b	1.1 ± 0.1aB	3.1 ± 0.1bB	0.38 ± 0.04aB
	30	2.4 ± 0.1a	1.1 ± 0.1aB	3.5 ± 0.1aB	0.35 ± 0.03aB
Virgin	15	0	3.8 ± 0.2bA	3.8 ± 0.2bA	1aA
	20	0	3.3 ± 0.3bA	3.3 ± 0.3bA	1aA
	25	0	4.9 ± 0.3aA	4.9 ± 0.3aA	1aA
	30	0	4.8 ± 0.2aA	4.8 ± 0.2aA	1aA
			ANOVA *F* values		
Temperature (Temp)		7.97 ***[a]	15.01 ***	13.01 ***	9.05 ***
Mating status (Mate)		-	615.92 ***	49.18 ***	3758.23 ***
Temp x Mate		-	8.38 ***	7.04 **	3.59 *

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA, $\alpha = 0.05$).

Mean progeny numbers or sex ratio followed by the same small letter are not significantly different among temperature treatments within a mating status. Mean rogeny numbers or sex ratio followed by the same capital letter are not significantly different between mating status within a specific temperature (Tukey's HSD, $\alpha = 0.05$).

a Only temperature effect on female progeny production by mated reproductive female was analyzed by one-way ANOVA.

Table 2.4 Means (± SEM) of longevity (in days) of female and male *Anagyrus loecki* adults subjected to various feeding treatments at five constant temperatures.

Temperature °C	Feeding treatment	Longevity	
		Female	Male
15	Starvation	3.9 ± 0.2bA	4.4 ± 0.3bA
	Distilled Water	6.8 ± 0.3bA	5.3 ± 0.6bA
	Honey Solution	52.8 ± 4.6aA	32.2 ± 3.6aA
20	Starvation	2.5 ± 0.1bB	2.2 ± 0.2bB
	Distilled Water	3.3 ± 0.2bAB	3.5 ± 0.2bB
	Honey Solution	32.5 ± 1.2aB	20.5 ± 1.2aB
25	Starvation	1.9 ± 0.1bC	1.8 ± 0.2bBC
	Distilled Water	2.5 ± 0.1bBC	2.3 ± 0.2bC
	Honey Solution	23.4 ± 1.7aBC	15.7 ± 2.8aB
30	Starvation	1.6 ± 0.1bC	1.7 ± 0.1bBC
	Distilled Water	1.8 ± 0.1bCD	2.1 ± 0.1bC
	Honey Solution	15.8 ± 1.1aCD	9.1 ± 1.0aC
35	Starvation	1.2 ± 0.1bC	1.2 ± 0.1bC
	Distilled Water	1.2 ± 0.1bD	1.3 ± 0.1bC
	Honey Solution	6.5 ± 0.8aD	3.9 ± 0.3aD
ANOVA *F* values			
Temperature (Temp)		68.92 ***	119.57 ***
Feeding treatment (Feed)		549.44 ***	301.66 ***
Temp x Feed		38.28 ***	39.38 ***

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA, $\alpha = 0.05$).

Mean longevity of females or males followed by the same small letter are not significantly different among feeding treatments within a specific temperature. Mean longevity of females or males followed by the same capital letter are not significantly different among temperature within a specific feeding treatment (Tukey's HSD, $\alpha = 0.05$).

Table 2.5 Regression coefficients (r^2) and *p*-values for the linear regression analyses between tibial length and longevity of female and male *Anagyrus loecki* adults subjected to various temperature/feeding treatment combinations.

Sex	Feeding treatment	Temperatures (°C)									
		15		20		25		30		35	
		r^2	*p*-value	r^2	*p*-value	r^2	*p*-value	r^2	*p*-value	r^2	*p*-value
Female	Starvation	0.0970	0.0881	0.3297	0.0002	0.1034	0.0831	0.1857	0.0174	0.1295	0.2062
	Water	0.1850	0.0157	0.2015	0.0214	0.2353	0.0120	0.2644	0.0051	0.0379	0.5660
	Honey	0.0449	0.2698	0.0695	0.0880	0.0530	0.2793	0.2057	0.0200	0.0865	0.3294
Male	Starvation	0.1557	0.2023	0.1562	0.1297	0.0001	0.9725	0.1894	0.1049	0.3792	0.0001
	Water	0.5485	0.0143	0.1942	0.0999	0.0010	0.9354	0.2522	0.0803	0.0532	0.1968
	Honey	0.1758	0.1993	0.0586	0.1612	0.9442	0.0057	0.0679	0.3681	0.0438	0.3158

Table 2.6 Longevity, reproductive period, lifetime fecundity and sex ratio (means ± SEM) of female *Anagyrus loecki* of different mating status at 25 °C.

Mating status	Longevity (days)	Reproductive period (days)	Number of offspring			Sex Ratio (Proportion of males)
			Female	Male	Total	
Virgin	12.7 ± 1.1b	9.6 ± 1.2b	-	44.0 ± 14.5a	44.0 ± 14.5b	1a
Male present 24 h	15.9 ± 1.3a	14.9 ± 0.3a	54.7 ± 8.9a	24.0 ± 5.0b	78.7 ± 13.1a	0.30 ± 0.03b
Male always present	15.9 ± 1.1a	15.7 ± 0.9a	52.0 ± 6.3a	25.0 ± 6.7b	77.0 ± 11.9a	0.31 ± 0.03b
			ANOVA *F* values			
Mating status	7.73 **	44.70 ***	0.06 NS	4.54 *	7.31 **	2657.25 ***

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA, $\alpha = 0.05$).

Means followed by the same letter within each column are not significantly different among mating status (Tukey's HSD, $\alpha = 0.05$).

Table 2.7 Longevity, reproductive period, lifetime fecundity and sex ratio (means ± SEM) of female *Anagyrus loecki* subjected to various feeding treatments at 25 °C.

Feeding treatments	Longevity (days)	Reproductive period (days)	Number of offspring			Sex Ratio (Proportion of males)
			Female	Male	Total	
Starvation	2.6 ± 0.4b	1.2 ± 0.4b	3.9 ± 1.6b	2.2 ± 1.1b	6.1 ± 2.5b	0.42 ± 0.15
Distilled water	4.0 ± 0.6b	2.3 ± 0.4b	7.2 ± 1.8b	3.0 ± 1.0b	10.2 ± 2.4b	0.30 ± 0.06
Honey solution	15.0 ± 2.1a	6.4 ± 0.7a	28.0 ± 5.3a	14.2 ± 2.1a	42.2 ± 7.3a	0.35 ± 0.02
			ANOVA *F* values			
Feeding treatment	46.07 ***	24.46 ***	15.41 ***	15.29 ***	18.43 ***	0.89 NS

***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA, $\alpha = 0.05$).

Means followed by the same letter within each column are not significantly different among feeding treatments (Tukey's HSD, $\alpha = 0.05$).

Figure Legends

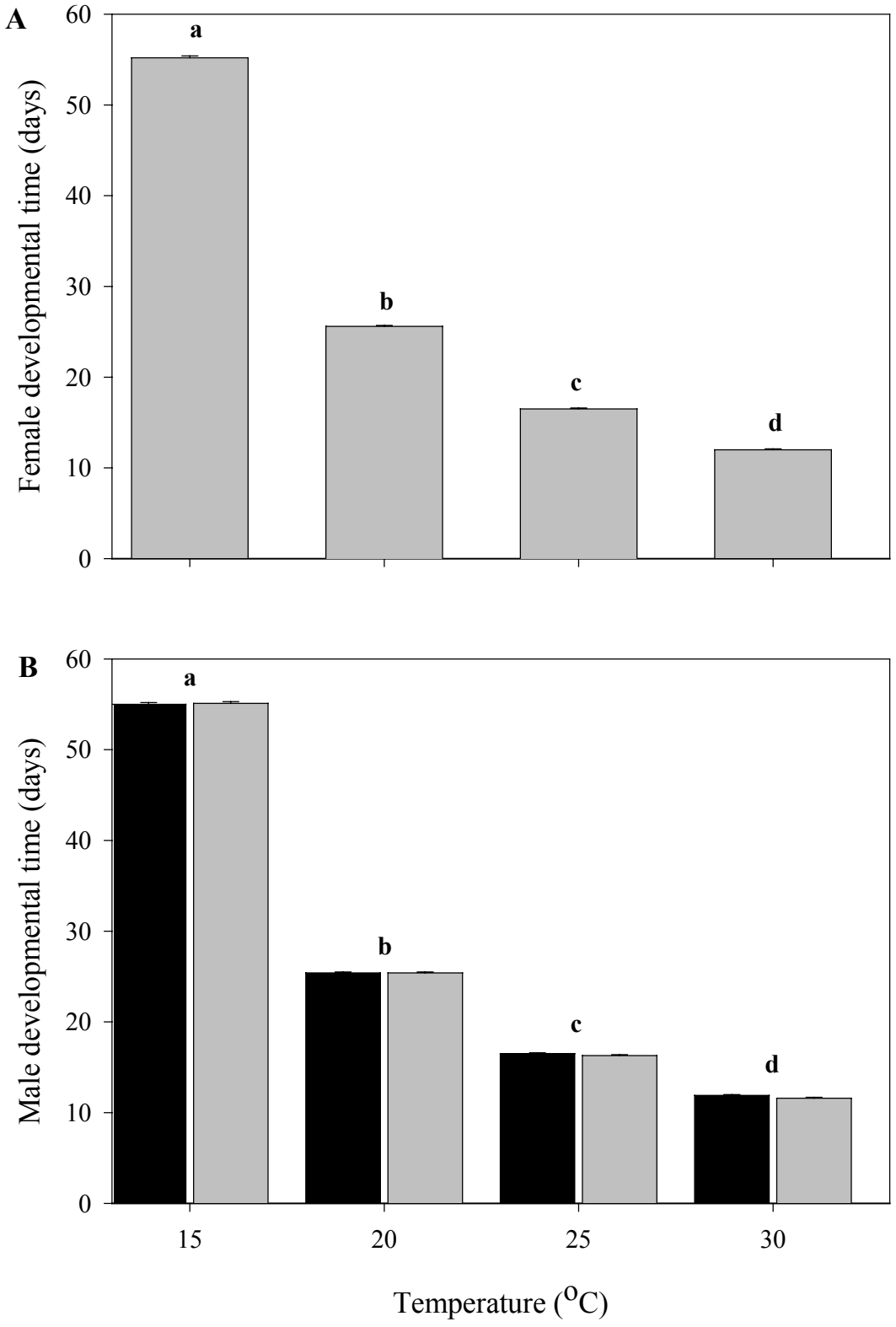
Fig. 2.1 Mean developmental times of female (A) and male (B) *Anagyrus loecki* produced by mated (grey bars) or virgin (black bars) females at various temperatures. Bars topped by the same letters are not significantly different.

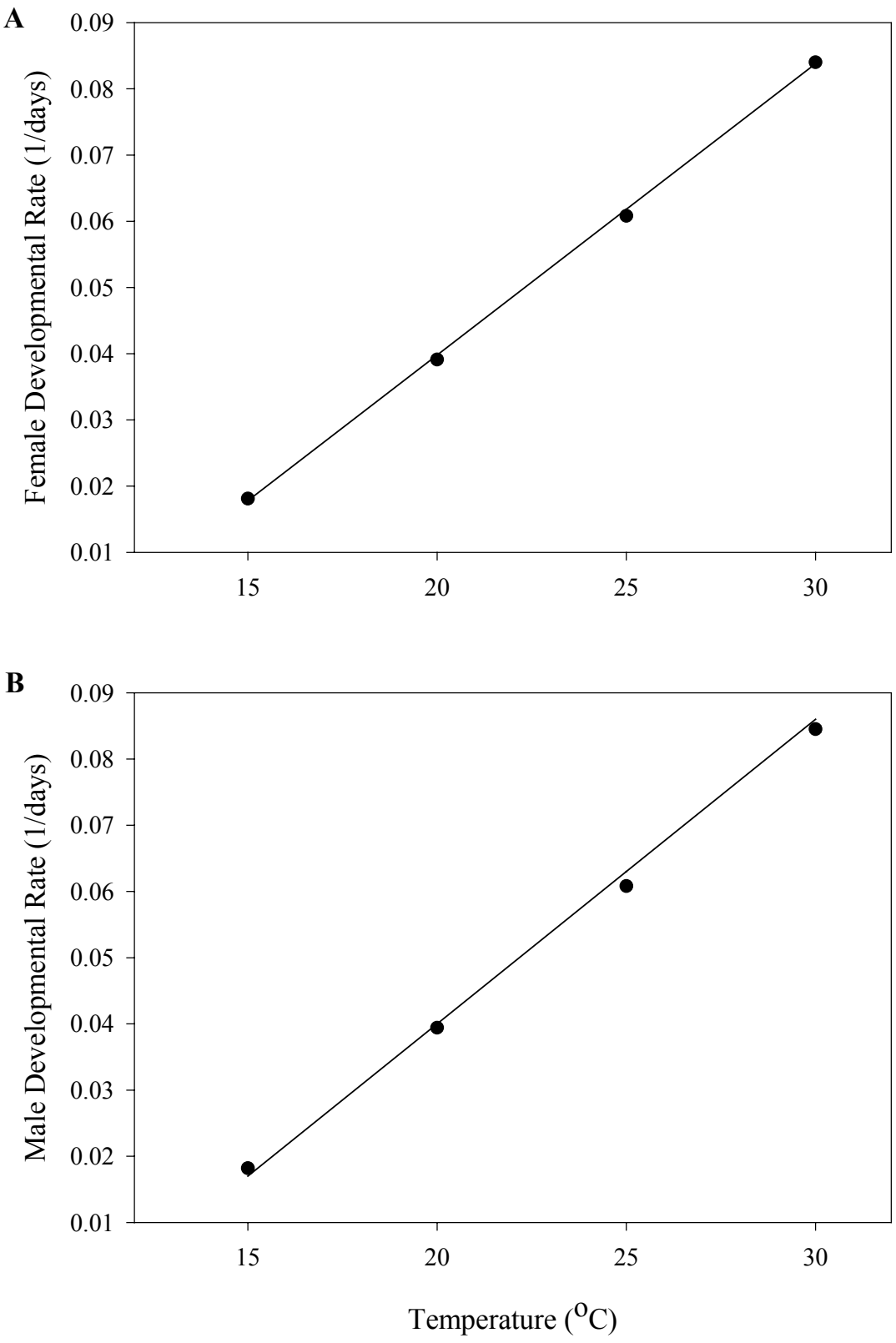
Fig. 2.2 Developmental rates (1/days) as a function of temperature for female (A) and male (B) *Anagyrus loecki*. The solid lines are regression fitted to the thermal summation equation.

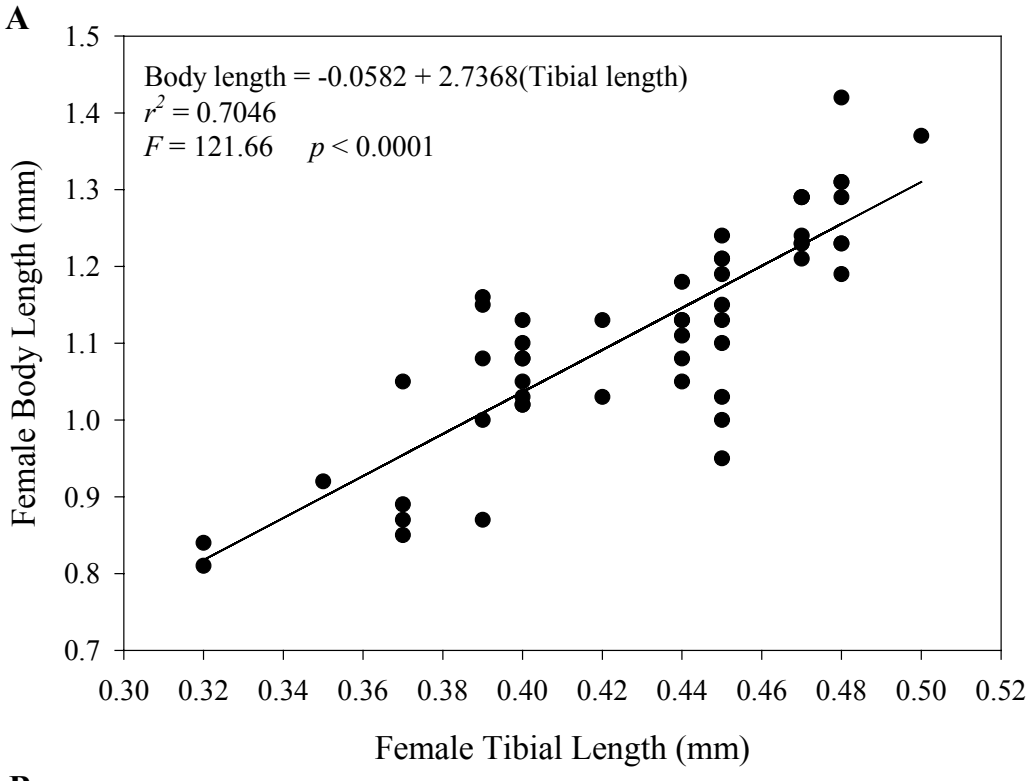
Fig. 2.3 Body length (mm) of female (A) and male (B) *Anagyrus loecki* as a function of hind tibial length (mm). The solid lines are the results of regression analyses.

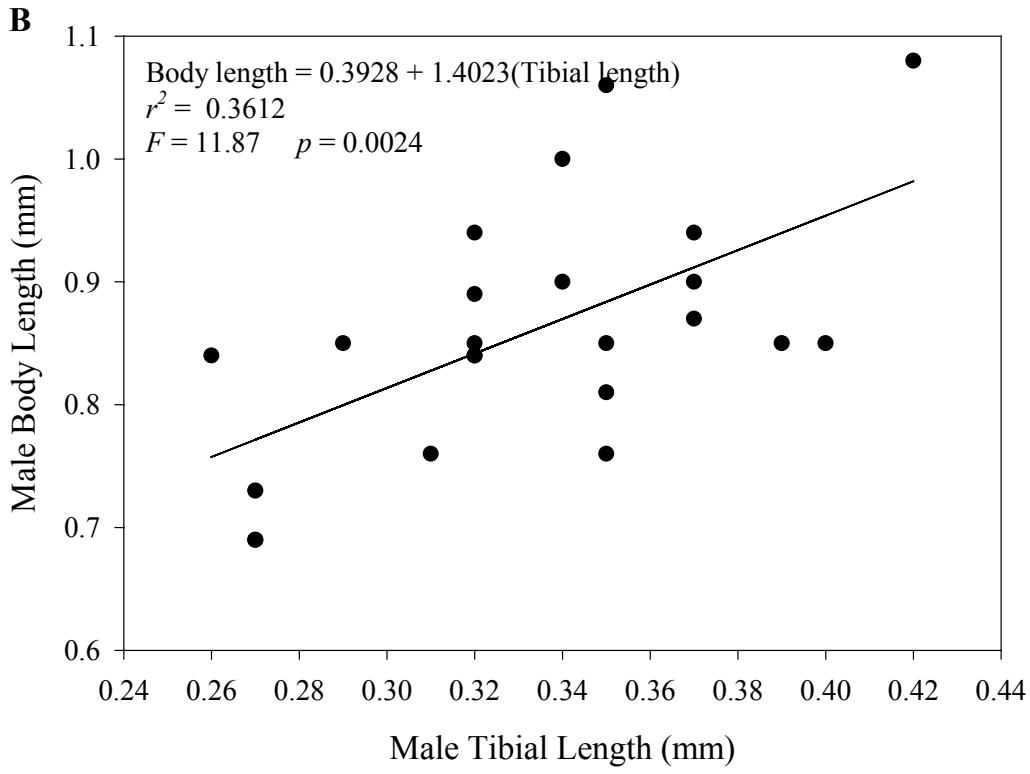
Fig. 2.4 Total number of progeny per day (A) and progeny sex ratio (B) produced by *Anagyrus loecki* of three mating status (virgin, ▼; mated for 24 h, ○; continuous male presence, ●) at 25 ^{o}C.

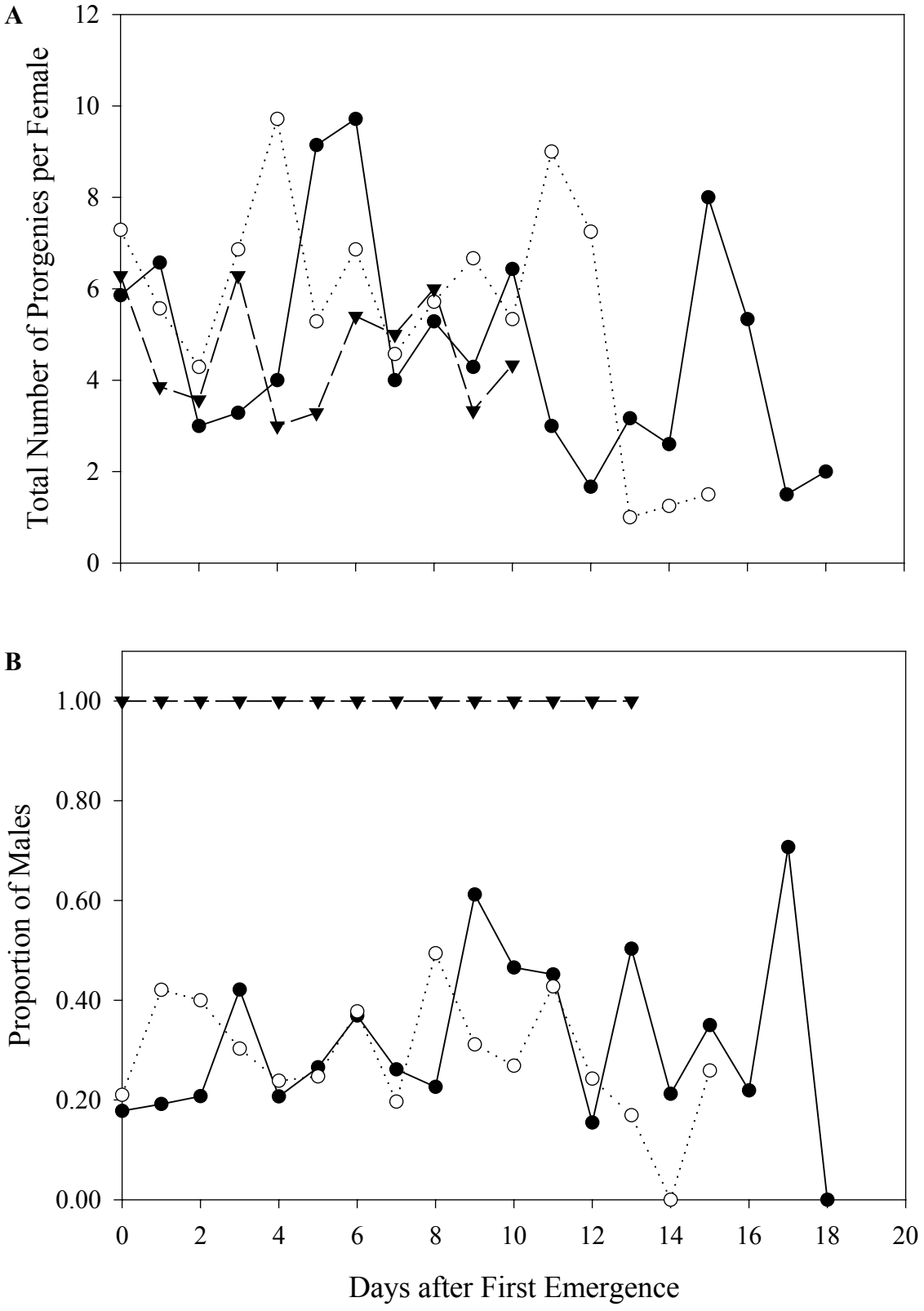
Fig. 2.5 Total number of progeny per day (A), brood sizes (B), and progeny sex ratio (C) produced by *Anagyrus loecki* subjected to three feeding treatments (50% honey solution, ▼; distilled water, ○; starvation, ●) at 25 ^{o}C.

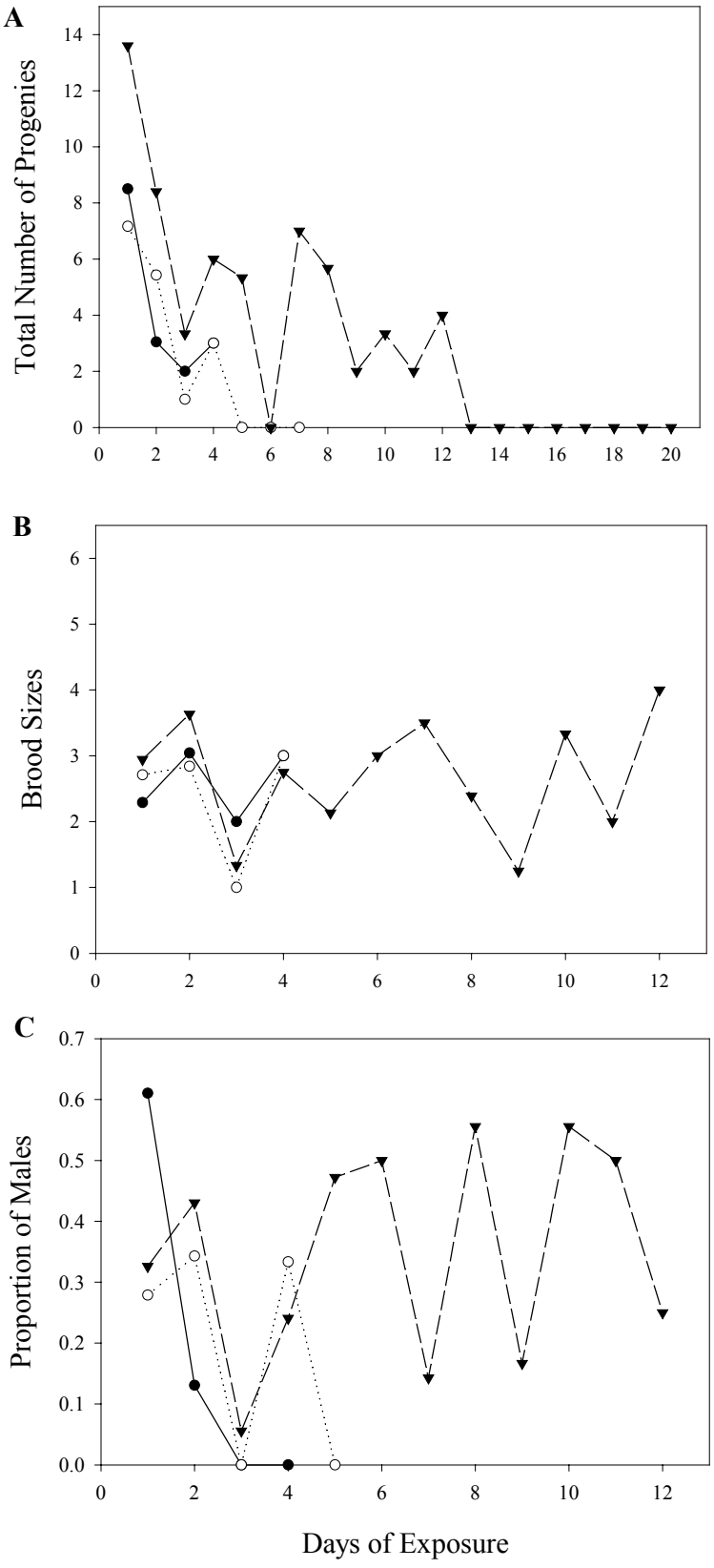

A
Female developmental time (days)
60
50
40
30
20
10
0
a
b
c
d
B
Male developmental time (days)
60
50
40
30
20
10
0
a
b
c
d
15
20
25
30
Temperature (°C)


A
Female Developmental Rate (1/days)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
15
20
25
30
B
Male Developmental Rate (1/days)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
15
20
25
30
Temperature (°C)


A
Body length = -0.0582 + 2.7368(Tibial length)
r^2 = 0.7046
F = 121.66 p < 0.0001
Female Body Length (mm)
Female Tibial Length (mm)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.30 0.32 0.34 0.36 0.38 0.40 0.42 0.44 0.46 0.48 0.50 0.52


B
Body length = 0.3928 + 1.4023(Tibial length)
r^2 = 0.3612
F = 11.87 p = 0.0024
Male Body Length (mm)
Male Tibial Length (mm)
1.1
1.0
0.9
0.8
0.7
0.6
0.24 0.26 0.28 0.30 0.32 0.34 0.36 0.38 0.40 0.42 0.44


A
Total Number of Prorgenies per Female
0
2
4
6
8
10
12
B
Proportion of Males
0.00
0.20
0.40
0.60
0.80
1.00
0
2
4
6
8
10
12
14
16
18
20
Days after First Emergence


A
Total Number of Progenies
0
2
4
6
8
10
12
14
0
2
4
6
8
10
12
14
16
18
20
B
Brood Sizes
0
1
2
3
4
5
6
0
2
4
6
8
10
12
C
Proportion of Males
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
2
4
6
8
10
12
Days of Exposure

CHAPTER 3

HOST STAGE PREFERENCE AND SUITABILITY BY THE MEALYBUG PARASITOID *ANAGYRUS LOECKI* (HYMENOPTERA: ENCYRTIDAE)[1]

[1] Chong, J.-H., and R. D. Oetting. 2005. To be submitted to the Annals of the Entomological Society of America.

ABSTRACT *Anagyrus loecki* Noyes & Menezes (Hymenoptera: Encyrtidae) was evaluated as a biological control agent of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). In this study, the effects of host developmental stages (first-, second-, male and female third-instar nymphs, pre-reproductive adult females, and ovipositing females) on the oviposition behaviors, parasitism, development, survival, sex allocation and progeny fitness of *A. loecki* was investigated. *Anagyrus loecki* parasitized mealybugs of all developmental stages but showed preference for third-instar immature females and pre-reproductive adult females when all host stages were offered simultaneously. The highest number of eggs was deposited in the third-instar immature females. *Anagyrus loecki* developed and emerged from hosts of all developmental stages. Third-instar and young adult females were the most suitable host stages for the development of *A. loecki*, with the progeny emerged from these hosts exhibiting shortest developmental time, highest survival rates, and largest progeny body size. In addition, more female progeny and larger broods emerged from older hosts. As a koinobiont parasitoid, *A. loecki* allowed the continuous growth of mealybugs after parasitism. The host stage at which mummification occurred had a significant influence on the development, brood size, sex allocation and progeny quality of *A. loecki*. We suggest that host quality should not be evaluated solely on the host stage at the time of parasitism, but also on the potential of young hosts to grow into later and more suitable developmental stages.

KEY WORDS *Anagyrus loecki*, Encyrtidae, *Phenacoccus madeirensis*, Pseudococcidae, host stage selection

Successful parasitism by parasitoids is often divided into five steps: host habitat selection, host location, host acceptance, host suitability and host regulation (Vinson 1976). Understanding parasitoid foraging behavior is important to the study of parasitoid life history, host-parasitoid interaction, population dynamics and community structures. Considerable effort has been made in studying parasitoid foraging and oviposition behavior (e.g., reviews in Vinson 1976; Godfray 1994).

In an aggregated host patch, such as that of a mealybug population, a parasitoid often encounters simultaneously hosts of different developmental stages. These hosts often differ in age and body size, and thus represent resources of varying qualities and quantities. The nutritional quantity of a host is determined by the amount of host tissues available for parasitoid larval development and the host quality is dependent upon the hosts' behavioral and immunological defenses (Vinson and Iwantsch 1980). Host selection by a parasitoid significantly influences the development, survival, sex allocation and fitness of progeny.

For idiobiont parasitoids, which often kill or paralyze the attacked hosts, the amount of resource available for progeny development is determined by the host body size at the time of parasitism. As a result, solitary idiobiont parasitoids are expected to attack larger hosts, which contain a greater quantity of resources, than small hosts. Progeny that emerge singly from larger hosts benefit from increased adult size that tend to positively correlated to fitness parameters, such as fecundity and survival of the parasitoids (Mackauer and Sequeira 1993). Koinobiont parasitoids, on the other hand, may exhibit a wider range of acceptable host sizes or host stages because the small hosts, although parasitized, may continue to develop and acquire resources for the development of parasitoid larvae. However there are costs involved in the parasitism of

small hosts: developmental time is often lengthened and survival is often reduced (Godfray 1994).

Selection of the most profitable host stage also influences the sex allocation patterns in arrhenotokous parasitoids. A higher proportion of females may be produced from larger hosts because of the greater nutritional requirement and reproductive benefits for the female progeny (Charnov et al.1981). Gregarious parasitoids have to make an additional decision on clutch size in relation to host developmental stage. The number of eggs deposited per host is expected to increase with host size or developmental stage (Godfray 1994), and based on the expected growth of the parasitized hosts. There is a trade-off between the number and quality of progeny of a gregarious parasitoid: the quality of progeny may decrease as the level of competition increases with the number of progeny per host (Godfray 1994).

Host stage preference and suitability by a parasitoid also have practical implications in biological control programs. Augmentative release requires mass rearing of a large number of high-quality biological control agents in the insectary. An understanding of the host stage selection by a parasitoid allows the manipulation of host stage composition in the insectary to yield biological control agents of suitable quality and quantity. Release of the parasitoids could also be synchronized with the phenology of the pests so that the most suitable host stages are available for parasitism at the time of release. A well-timed release could achieve a higher level of control and better chance of establishment. Comparisons of the host stage preference of different candidate species that utilize the same host species will allow the design of multiple-species introduction programs that may minimize competition among the released biological control agents.

Anagyrus loecki Noyes & Menezes is a gregarious koinobiont parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* Green, a common pest species in greenhouse ornamental production of the Southeastern United States. This encyrtid parasitoid is a candidate species for augmentative release against the Madeira mealybugs. The objectives of this study were to investigate the influences of host developmental stages on the foraging decisions, parasitism, development, survival, progeny production, sex allocation pattern, and progeny quality of *A. loecki*. Specifically, the following hypotheses were tested: *A. loecki* will prefer older and larger hosts for oviposition; more advanced host stages will yield progeny of higher fitness; sex ratio of the parasitoid will be male-biased in younger hosts and female-biased in older hosts; and increasing brood sizes will negatively affect parasitoid development and body size.

Materials and Methods

Maintenance of Insect Cultures. The Madeira mealybugs were reared on sprouted russet potatoes (*Solanum tuberosum* L.) in an insectary at the University of Georgia, Griffin Campus, Griffin, GA. The insectary was maintained at a temperature range of 25-28^{o}C and a photoperiod of 16 h. To establish mealybug colonies of uniform developmental stage, each week separate groups of sprouted potatoes were infested with 100 ovisacs collected from an existing Madeira mealybug colony. Individuals of the following developmental stages were collected for the experiments: crawlers (first-instar nymphs), second-instar nymphs, third-instar immature females, third- or fourth-instar males wrapped in tests, pre-reproductive adult females and reproductive females with 2-d-old ovisacs. Within each developmental stage, mealybugs of similar sizes were selected to minimize quality differences due to body size.

Laboratory colonies of *A. loecki* were established using cultures received from the University of Florida, Mid-Florida Research and Education Center, Apopka, FL in September

2000. Numerous generations had been maintained on Madeira mealybugs reared on sprouted potatoes in the laboratory before the start of this study. Mummies were collected from the laboratory colonies, isolated in individual gelatin capsules (no. 1, Eli Lilly and Co., Indianapolis, IN) and held at 25°C until adult emergence. Within 24 h of emergence, the adult parasitoids were transferred into plastic vials with droplets of diluted honey solution as food, and held at 25°C for 72 h before the experiments. Each female parasitoid was paired with 2 males to ensure mating. No hosts were provided during the holding period so the parasitoids were naïve at the start of the experiments.

Oviposition Behaviors and Host Stage Preference Study. Two types of experiments were designed to study the oviposition behaviors of *A. loecki*: no-choice tests in which the parasitoids were provided with mealybugs of a particular developmental stage, and choice tests in which the parasitoids were allowed to choose the most preferred hosts from a population of mealybugs of mixed developmental stages. The observation arenas used in the choice and no-choice tests were constructed with 35-mm petri dishes. A coleus (*Solenostemon scutellarioides* Thonn.) leaf disk was cut to fit the size of the petri dish, and was placed underside up in the petri dish. Wetted filter papers lined the bottom of the petri dish to prevent desiccation of the leaf disk.

In the no-choice tests, 10 individual mealybugs from one of the six host stages were collected from the mealybug colony and transferred onto each coleus leaf disk with a fine paint brush. In the choice tests, 2 individuals from each of the five development stages (all except the third- or fourth-instar immature males) were introduced into the observation arenas. Mealybugs from all developmental stages were prepared and allowed to settle on the leaf disk 16 h before the experiments, except for the ovipositing females and immatures males. To prepare the ovipositing adult females, mature adult females that were starting to construct ovisacs were

collected before egg production and isolated in their respective observational arenas 2 d before the start of the experiments. Third or fourth-instar males were grouped into a single developmental stage for the experiments. The immature males were prepared by collecting late second-instar nymphs, which showed pink coloration, and transferring them into the observational arenas 2 d before an experiment. The position of each mealybug was marked on a piece of paper to facilitate subsequent identification of the mealybug during the behavioral observation and video recordings.

The foraging behaviors of *A. loecki* were studied under laboratory conditions of 21-25^{o}C, 45-70% relative humidity and artificial fluorescent lighting. Based on previous behavioral studies on mealybug parasitoids of the Encyrtidae (e.g. Bokonon-Ganta et al. 1995, Karamaouna and Copland 2000b, Joyce et al. 2001) and on the preliminary studies on *A. loecki*, we prepared a list of expected behaviors and the characteristic of each behavior (Table 3.1). The behaviors include: searching, grooming, resting, antennal examination, ovipositor probing, oviposition, host feeding, and water and honeydew feeding. Since an encounter was always followed by an antennal examination, encounter and examination were grouped into a single behavior type called 'examine'. The parasitoids appeared to continue antennal examination while they were mounting the hosts. Thus the duration of mounting was pooled into the duration of antennal examination. Mealybugs often exhibit defensive behaviors upon encounter with the parasitoids, thus we also prepared a list of expected host defensive behaviors, which include walk away, abdomen flipping, and reflex bleeding.

Observation began when a female *A. loecki* was released into the observational arena. The foraging behavior of the parasitoid and the response of the mealybugs were continuously observed and filmed with a solid-state color video camera (Hitachi, Japan) fitted with an 18-

108/2.5 zoom lens (Computar, Japan) for 60 min. The foraging behaviors of 140 parasitoids, 20 individuals for each host developmental stage in the no-choice tests and a total of 20 individuals for the choice tests, were observed. The frequency and duration of each behavioral event were recorded with the behavior observation program Observer® (version 4.1, Noldus Information Technology, Wageningen, the Netherlands). The stung mealybugs were not removed from the arena, thus allowing the parasitoids to superparasitize some individuals.

The proportion of hosts parasitized and the time searching in a host patch indicated the preference of a parasitoid. The observational arenas were not covered, allowing the parasitoids to determine their residence time (up to 60 min) and leave the arena when needed. An observation was terminated at the end of the 60-min observation period, or when the parasitoid did not exhibit any foraging behavior for more than 10 min, or when the parasitoid left the arena. If a parasitoid was ovipositing in a host at the end of the 60-min observation period, it was allowed to complete the behavioral event and the observation was terminated after the parasitoid withdrew its ovipositor and left the host. In this case, the additional observation time was added to the total observation duration.

To verify successful egg deposition and to determine the clutch sizes, the stung mealybugs were removed 3-24 h after the observation period, dissected in a drop of distilled water on a microscope slide and examined at 100X magnification. The parasitoid eggs were released into the dissecting fluid when the host body was ruptured and appeared oblong with a short stalk, which made them distinguishable from the larger rounded mealybug eggs.

The frequency and duration of each behavioral event were tallied using the Observer program and used in the construction of a behavioral sequence and time budget for *A. loecki* oviposition. Encounter rates of each host developmental stage in the no-choice tests were

calculated by dividing the frequency of antennal examination with the total duration of searching. The encounter rates were not calculated for the choice tests because searching time could not be accurately attributed to a particular host stage. In both no-choice and choice tests, the proportions of hosts examined, probed and oviposited were determined and arcsine-transformed before statistical analyses. The most preferred host stage had the highest proportion of total available hosts successfully parasitized by *A. loecki*. The influence of host stage on the proportion of hosts subjected to antennal examination and oviposition, and on the clutch sizes was analyzed with a one-way Analysis of Variance (ANOVA, PROC GLM, SAS 1999). When significant host stage effects were detected by ANOVA, the means were separated by Tukey's honestly significant difference (HSD) test at a significant level of 0.05. To determine if superparasitism altered egg distribution, the clutch sizes of superparasitized and parasitized hosts were analyzed by ANOVA and separated by Tukey's HSD test.

Host Stage Suitability Study. No-choice and choice tests were prepared with identical host stage composition, numbers, and procedure as in the host stage preference experiments. The rearing units used in the suitability experiments were constructed from 100 by 20 mm petri dishes. Each rearing unit contained an excised whole coleus leaf with the petiole inserted through a hole in the bottom of the petri dish and into a cup of water. The petri dishes were covered with fine-mesh chiffon to allow ventilation and prevent escape of the parasitoids and the mealybugs. Seventy-two-h-old naïve parasitoids were released individually into the rearing units and were allowed to forage for 24 h at 25^{o}C. After removal of the parasitoids, the mealybugs were held at 25^{o}C in the rearing units for 30 d. During this incubation period, the mealybugs were examined every 5 d and mummies were collected and isolated in individual gelatin capsules. The host stage at which the mealybugs were killed by the developing parasitoid larvae

and mummified were determined by the number of exuviae and verified by the developmental stages of the unparasitized mealybugs within the same rearing unit. The mummies were incubated at 25^{o}C until adult parasitoid emergence. Upon emergence the parasitoids were counted and sexed. All emerged parasitoids were anesthetized with carbon dioxide and their hind tibial lengths were measured (as a surrogate for the parasitoid body size) with a micrometer at 60X magnification. All mummies were dissected to verify the survival of parasitoid larvae or pupae. The choice tests and each developmental stage in the no-choice tests were replicated 60 times.

Preliminary statistical analyses suggested that the developmental time of male and female *A. loecki*, the hind tibial length, the brood size and the progeny sex ratio from the same host stage treatment were similar between the choice and no-choice tests. As a result, the data were combined and used for statistical analyses. The parasitism rates (proportion of hosts parasitized), developmental time, emergence rates (proportion of progeny emergence), brood size (number of progeny emerged per mummy), sex ratio (proportion of males) and hind tibial lengths were calculated for each host developmental stage at the time of exposure and the time of mummification. Parasitism rates, emergence rates and sex ratio were arcsine-transformed before statistical analyses to fit the data to a normal distribution and equalize data variance. Because the host stage at which the parasitized mealybug mummified depended on the exposed host stage, the effects of the two factors on the above calculated parameters were analyzed separately with one-way ANOVA (SAS 1999). Means were separated by Tukey's HSD test at a significant level of 0.05. The effects of brood size on developmental time and progeny hind tibial lengths were examined with Spearman's correlation (PROC CORR, SAS 1999).

Results

Oviposition Behaviors of *A. loecki*. The sequence of oviposition behavior of *A. loecki* in host populations of uniform developmental stage, based on the observations made in the no-choice tests, are presented in Fig. 3.1. In both the no-choice and choice tests, the total observation time for most mealybug developmental stages exceeded 60 min (Fig. 3.2). The parasitoids foraged for an average of 22 min in a population of immature males and they rejected all encountered hosts (Fig. 3.1D).

A female *A. loecki* started to search without apparent direction immediately after its introduction into the observation arena. The searching behavior was terminated when the parasitoid rested, groomed, fed or encountered a host. Few individuals rested during the observation period. The parasitoids often interrupted searching to feed on free-standing water or host honeydew droplets on the leaf surface. Host feeding was not observed in this study.

In the no-choice tests with ovipositing female mealybugs and first-instar nymphs, parasitoids spent more than 20% of the total observation time searching (Fig. 3.2A). The percentage of total time used for searching increased to 50% for parasitoids released into populations of immature males. The average searching time per event of *A. loecki* in the no-choice tests was longest for parasitoids with first-instar nymphs and ovipositing adult female mealybugs (Table 3.2). It took only an average of 0.30 min (lowest of all host stages) for a parasitoid that was searching in populations of pre-reproductive mealybugs to encounter a host or engage in other behavior.

The average probability of encountering a host of any developmental stage by a searching female *A. loecki* was 0.60 (Fig. 3.1). The encounter rates differed among the six host stages: the parasitoids encountered more older hosts than younger hosts per unit time (ANOVA, $F = 8.03$, P

< 0.0001). The pre-reproductive adult female and the immature male mealybugs were encountered at a rate of more than 3 hosts per min (Fig. 3.3). Only 1 first-instar nymph was encountered by the parasitoid every minute.

An encounter always led to prolonged antennal examination of the host. Female *A. loecki* appeared to spend more time examining older hosts: an average of 0.30 min for pre-reproductive adult females and 0.48 min for first-instar nymphs (Table 3.2). The probability of antennal rejection, which occurred when the female parasitoid left the examined host and continued to search for other hosts, was highest for third-instar immature female mealybugs (0.80) but similar for hosts of other developmental stages (around 0.40) (Fig. 3.1). Except for the crawlers, which were much smaller than the parasitoids, mealybugs of other developmental stages were mounted by the parasitoids (Fig. 3.1).

If a female *A. loecki* accepted a host after antennal examination, it turned around and attempted to insert its ovipositor. The parasitoid often probed the mealybug on the lateral side if the parasitoid stood on the leaf surface or on the dorsum if the host was mounted. Some parasitoids made several attempts to insert their ovipositors. The mean duration for probing (45 s to 1 min) was similar among the six developmental stages (Table 3.2). Some mealybugs were rejected after several failed attempts to insert the ovipositor. The rejections were due to either host defense, missing the mealybug or repeated failure to insert the ovipositor in an appropriate area of the host's body. Most parasitoids proceeded to oviposition after successful ovipositor insertion, except for those attacking the immature male mealybugs. Although immature males were probed, no ovipositing behavior (characterized by prolonged ovipositor insertion with motionless antennae or body) was observed. Subsequent dissections did not recover eggs from the probed immature males.

Oviposition occupied more than half the total observation time (Fig. 3.2) and the mean durations were different among the host developmental stages in both the no-choice and choice tests (Table 3.2). The longest average oviposition period was observed in parasitoids attacking the third-instar immature female mealybugs (15 min) and the shortest in those attacking the crawlers (5-6 min) (Table 3.2). The average oviposition time for other developmental stages ranged between 8-11 min. On average, more than 94% of the parasitoids walked rapidly away from the mealybugs when oviposition was completed and they proceeded to search for a new host (Fig. 3.1). Sometimes the parasitoids stopped and groomed for 10-15 s before resuming host searching.

A searching parasitoid may encounter a host that was previously parasitized. Some of these parasitized mealybugs were accepted for superparasitism. In the choice tests, the proportions of superparasitized hosts (0.12-0.25) were similar among the five host stages. The superparasitism rates were significantly different among the development stages in the no-choice tests (ANOVA, $F = 3.11$, $P = 0.0118$), which were 0.08, 0.14, 0.31, 0.27 and 0.33 for first-instar nymphs, second-instar nymphs, third-instar immature females, pre-reproductive adult females and ovipositing females, respectively.

Mealybug Defensive Behaviors. The Madeira mealybugs defended themselves by walking away from the parasitoid or by flipping their abdomens. No reflex bleeding was observed. Antennal examination, probing and oviposition could provoke defensive behavior from the mealybugs. The proportions of encountered mealybugs exhibiting defensive behaviors were 0.08, 0.16, 0.08, 0.26, and 0.02 for the crawlers, second-instar nymphs, third-instar immature females, pre-reproductive adult females, and ovipositing female mealybugs, respectively. No immature males, which were wrapped in thick layers of wax filaments, defended themselves

from parasitoid attacks. A higher proportion of older hosts defended themselves (mean = 0.26; ANOVA, $F = 4.66$, $P = 0.0007$) and had a better chance of fending off parasitoid attacks (ANOVA, $F = 9.87$, $P < 0.0001$). On average, only 3, 9, 5, 9 and 0% of crawlers, second-instar nymphs, third-instar nymphs, pre-reproductive adults and ovipositing adults, respectively, that exhibited defensive behavior caused the parasitoids to retreat. The majority of encountered mealybugs were unable to deter attacks by the parasitoids. Even when a mealybug walked away from its original feeding site the parasitoid pursued the mealybug until it encountered another mealybug, which it promptly examined, or succeeded in parasitizing the pursued host.

Host Stage Preference and Clutch Sizes. Preference for a particular host stage was signified by a higher proportion of hosts parasitized compared to the proportion of the hosts encountered. In the no-choice tests, the proportion of mealybugs encountered by the parasitoids was similar among all host stages tested, except for the immature males (Table 3.3). The proportion of hosts examined by the parasitoids in the choice tests ranged from 0.2 for crawlers to 0.75 for pre-ovipositing females (Table 3).

More than 50% of the encountered and examined mealybugs were probed (Table 3.3). All developmental stages of the Madeira mealybug, except the immature males, were parasitized by *A. loecki* in both the choice and no-choice tests. In the no-choice tests, female *A. loecki* parasitized similar proportions of encountered and examined hosts from all host stages except the immature males. In the choice tests, *A. loecki* showed a clear preference for third-instar and pre-reproductive adult female mealybugs. Less than 30% of the encountered and examined first- and second-instar nymphs were parasitized.

Clutch sizes (the numbers of eggs deposited per mealybug) from the choice and no-choice tests were similar within a host stage (for all stages, $F < 1.0$, $P > 0.50$). Data from the

choice and no-choice tests were thus pooled for statistical analyses. The clutch sizes differed significantly among the five host stages attacked by *A. loecki* (Fig. 3.4; ANOVA, $F = 24.29$, $P < 0.0001$). The largest clutches were recovered from the third-instar immature females, with an average of 4 eggs per mealybug, followed by those in the pre-reproductive and ovipositing female mealybugs. Each crawler and second-instar nymph generally received only 1-2 eggs. Superparasitized hosts of a particular developmental stage contained additional 1-3 eggs per host compared to once-parasitized hosts (ANOVA, $F = 13.90$, $P = 0.0002$).

Host Stage Suitability. In the no-choice tests, *A. loecki* failed to parasitize any immature male mealybug, but successfully parasitized a similar proportion (0.3-0.4) of the total available mealybugs from all other host stages (ANOVA, $F = 26.50$, $P < 0.0001$; Fig. 3.5A). In the choice tests, third-instar immature female mealybugs were the most preferred hosts as evidenced by a higher parasitism rate than for any other host stages (ANOVA, $F = 30.28$, $P < 0.0001$; Fig. 3.5B). On average, more than 60% of the available third-instar female Madeira mealybugs were parasitized in the choice tests. In contrast, less than 10% of crawlers were parasitized in the same tests.

Anagyrus loecki successfully completed development in all host stages of the Madeira mealybug. In the no-choice tests, the progeny emergence rate ranged between 93 and 99% with the lowest emergence rate occurring in the ovipositing female mealybugs (ANOVA, $F = 10.75$, $P < 0.0001$; Fig. 3.6A). A similar trend was observed in the choice tests but the difference in progeny emergence rate among the five host developmental stages were not statistically significant (ANOVA, $F = 0.95$, $P = 0.4336$; Fig. 3.6B).

Madeira mealybugs parasitized by *A. loecki* continued to develop into more advanced developmental stages (Fig. 3.7). On average, 73% of parasitized crawlers continued

development to the second nymphal instar, 26% to third nymphal instar, and 1% to adulthood. Most second-instar nymphs developed to the third nymphal instar (mean = 32%) or adulthood (mean = 23%) before they were killed by the parasitoid larvae. More than 75% of the parasitized third-instar immature female mealybugs completed development to adulthood. The pre-reproductive female mealybugs continued to mature after parasitism and 50% of them reproduced before mummification. The ovipositing female mealybugs continued to deposit eggs until the parasitoid larvae consumed all mealybug tissues and killed them.

Host developmental stage at the time of parasitism also had significant impacts on *A. loecki* developmental time (Table 3.4). Parasitoids that were developing in the younger mealybugs emerged significantly later than those developing in the older mealybugs. The parasitoids that emerged from hosts parasitized as crawlers took more than 27 d to complete development. The parasitoids that developed in adult female mealybugs (both pre-reproductive and ovipositing) emerged in 16 d at 25 ^{o}C. The parasitoids completed development in 20 d in the mealybugs attacked as second- or third-instar nymphs at the time of parasitism.

Host stage at the time of mummification also significantly affected the developmental time of *A. loecki* (Table 3.4). Within each Madeira mealybug cohort that was parasitized at the nymphal stage, parasitoids emerged later from the mummies that had achieved a more advanced developmental stage than from younger mummies. Many crawlers continued to develop into second- or third-instar nymphs and adult mealybugs before mummification. Parasitoids emerged from the second-instar mummies in 27 d, which was 7 d shorter than the parasitoids emerged from the mummies that had achieved adult development. Some parasitized second-instar nymphs went on to complete adult development, and when parasitoids emerged from the adult mummies it took 9 d longer for the parasitoids to complete development than those that emerged

from the second-instar mummies. Parasitoids that emerged from pre-reproductive adult mummies and ovipositing adult mummies were similar in developmental time with an average of 16 d.

Significant correlations were detected between brood size and developmental time of *A. loecki*. Larger brood size was positively correlated with longer duration of development in hosts that were parasitized as crawlers, second-instar and third-instar nymphs. The Spearman's correlation coefficients were 0.3252, 0.5872 and 0.2512 (all $P < 0.0001$) for parasitoids that were developing in the immature mealybugs. Significant negative correlation (Spearman's correlation coefficient = -0.2391, $P = 0.0001$) was detected between brood size and developmental time of parasitoids developing in hosts attacked as pre-reproductive females. The correlation between brood size and developmental time was not significant for parasitoids that emerged from the ovipositing mealybugs (Spearman's correlation coefficient = -0.0222, $P = 0.8596$).

Anagyrus loecki is a gregarious parasitoid, meaning that more than 1 progeny emerged from each mummy. The brood size of *A. loecki* was significantly influenced by host stage at the time of oviposition (Table 3.5). Oviposition in the pre-reproductive adult female mealybugs produced the largest brood size (3 parasitoids per mummy), followed the third-instar immature female mealybugs (2.5 parasitoids per mummy). Mummies resulted from the ovipositing mealybugs and the first- and second-instar nymphs produced 1-2 parasitoids per mummy.

Host stage at the time of mummification also had a significant influence on the brood size of *A. loecki* (Table 3.5). Regardless of the host stage at the time of parasitism, older and larger mummies yielded a larger brood size. Only 1 or 2 individuals emerged from each mummy that had achieved either the second or third nymphal instar and the mummy resulted from ovipositing

mealybugs. The brood size increased to 3 parasitoids per mummy when the hosts completed development to adulthood before been killed by the parasitoid larvae.

The progeny sex ratio of *A. loecki* attacking the youngest and the oldest Madeira mealybugs at the time of parasitism were skewed toward males: 50% or more of the progeny that were produced from crawlers and ovipositing mealybugs were males (Table 3.6). The proportion of males was reduced to 0.34 and 0.36 when the hosts were attacked in the third nymphal instar and young adult stage, respectively.

The host stage at which mummification occurred also had significant influence on progeny sex ratio of *A. loecki* (Table 3.6). The parasitized second-instar nymphs produced progeny with proportion of males of 0.89 when mummified at the second nymphal instar, 0.22 when mummified at the third instar and 0.27 when mummified as adults. Although parasitized as third-instar nymphs, the mummies that had achieved adult development produced 6% more female parasitoids than the mummies that remained as third nymphal instar. Parasitoids produced equal proportions of male progeny in the pre-reproductive adult mealybugs, regardless of the stage of mummification.

The hind tibial lengths of female *A. loecki* ranged from 0.33 to 0.35 mm and was longer than that of the males (0.27-0.30 mm) (Table 3.7). The hind tibial length of both male and female parasitoids varied with host stage at the time of oviposition. Parasitoids of either sex with the longest tibial length (thus the largest body size) were produced from hosts parasitized as third-instar nymphs or pre-reproductive adult female mealybugs. The parasitoids produced from the crawlers were significantly smaller than those from the third-instar or pre-reproductive adult mealybugs.

Regardless of the host stage at the time of parasitism, the parasitoids that emerged from mummies that had achieved more advanced development were larger than those emerged from younger mummies (Table 3.7). The variance in body size was greatest in parasitoids developing in hosts parasitized as first- and second-instar nymphs. Although the parasitoid eggs were laid in first-instar nymphs, the adult parasitoids that emerged from mummies that attained the third nymphal instar were larger than those that emerged from second-instar mummies.

Negative correlations between sibling number and hind tibial length of *A. loecki* were detected in male and female parasitoids developing in hosts of most developmental stages (Table 3.8). The tibial length of female parasitoids was positively correlated to sibling numbers when the mealybugs were attacked as first-instar nymphs. A similar trend was observed in male parasitoids that emerged from mummies parasitized as second-instar nymphs. Strong negative correlations were detected in female and male parasitoids developing in mealybugs parasitized as third-instar and pre-reproductive adult mealybugs.

Discussion

Oviposition behaviors of *Anagyrus loecki*. *Anagyrus loecki* forages in a stereotypical sequence of behaviors described in other encyrtid mealybug parasitoids (Boavida et al. 1995, Bokonon-Ganta et al. 1995, Karamaouna and Copland 2000b, Joyce et al. 2001). Female *A. loecki* searched by drumming the leaf surface until encountering a host, which the parasitoid always examined with its antennae. Similarly, the parasitoid *Gyranusoidea tebygi* Noyes examined every mango mealybug (*Rastrococcus invadens* Williams) encountered (Boavida et al. 1995). In contrast, *Coccidoxenoides peregrinus* (Timberlake) sometimes walked over its hosts [*Planococcus ficus* (Signoret)] without any notable sign of recognition (Joyce et al. 2001). It is possible that the wax on the body surface of Madeira mealybug contains chemical cues important

in host recognition. It was observed that in some instances, wax filaments and residuals on the leaf surface elicited antennal examination and probing behaviors by *A. loecki*. This behavior suggests the presence of chemical cues for recognition.

Anagyrus loecki spent the majority of its foraging time ovipositing (more than 50%) and searching (20%). The proportion of total time spent in searching for hosts was reported to be 72% for *C. peregrinus*, while probing and oviposition occupied 5.6% of total time (Joyce et al. 2001). When data from all host stages were combined, *G. tebygi* spent 25 and 24 % of the total time on searching and attacking (examination and probing combined) hosts, respectively (Boavida et al. 1995). The average time for each oviposition event by *A. loecki* was over 6 min when attacking crawlers, and increased to 15 min for third-instar females. The average oviposition time reported in this study was much longer than that reported for *C. peregrinus* (28 s; Joyce et al. 2001). The difference may be due to the fact that *A. loecki*, as a gregarious parasitoid, requires more time to deposit multiple eggs whereas the solitary *C. peregrinus* terminates oviposition as soon as one egg is deposited. The phenomenon of increased mean oviposition time in larger hosts of a more advanced developmental stage was also observed in the solitary *G. tebygi* (Boavida et al. 1995) and *Anagyrus mangicola* Noyes (Bokonon-Ganta et al. 1995).

Host stage preference of *Anagyrus loecki*. Female *A. loecki* parasitized Madeira mealybugs of all developmental stages. The parasitism rate in the no-choice tests was similar among all host stages but slightly higher for the second-instar nymphs and pre-reproductive females. In the choice tests, *A. loecki* showed clear preference for third-instar and pre-reproductive female mealybugs. Even though crawlers were encountered and examined, they were parasitized at a lower rate. Most mealybug parasitoids of the Encyrtidae also showed preference for a range of

host stages or host sizes for parasitism. *Coccidoxinoides peregrinus* parasitized all developmental stages of the citrus mealybug, *Planococcus citri* (Risso) but showed preference for second- and third-instar nymphs (Ceballo and Walter 2004). Both *A. mangicola* and *Anagyrus kamali* Moursi attacked all developmental stages of their respective hosts but preferred the larger and more advanced host stages over the smaller and younger stages (Bokonon-Ganta et al. 1995, Sagarra and Vincent 1999, respectively). The narrowing of the range of preferred host stages was most pronounced in *Anagyrus indicus* Shafee et al. (Nechols and Kikuchi 1985). *Anagyrus indicus* parasitized hosts of all developmental stages in the no-choice tests. However, when given a choice of host stages *A. indicus* completely ignored the crawlers and second-instar nymphs and had a strong preference for adult mealybugs and to a lesser extent the third-instar nymphs of the spherical mealybug, *Nipaecoccus viridis* (Newstead).

Third- and fourth-instar male Madeira mealybugs were wrapped in thick waxy tests and may be protected from parasitism. In this study, *A. loecki* probed these immature males but no eggs were deposited. However, we observed that a second-instar male, which had not yet produce the waxy tests, was as likely to be attacked by the parasitoid as a female of the same stage. In a study of the host preference of *Anagyrus* (= *Epidinocarsis*) *diversicornis* (Howard), Van Driesche et al. (1987a) reported that the majority of examined third-instar males of the cassava mealybug, *Phenacoccus herreni* Cox and Williams were rejected for probing whereas the second-instar males were 10 times more likely to be probed by the parasitoids. In the same study, another mealybug parasitoid *Acerophagus coccois* Smith probed the same proportion of second- and third-instar males but almost no third-instar males were parasitized.

Many synovigenic parasitoids host feed to obtain proteins for egg production (Jervis and Kidd 1986). Hosts that are fed on are usually not suitable for egg deposition due to the depletion

of resources and death of the hosts. For a female parasitoid, the decision to host feed or oviposit depends on the host stage that was encountered and the female's egg load (Rosenheim and Rosen 1991). Based on the observations on *Aphytis* species (Hymenoptera: Aphenilidae), Luck et al. (1982) and Rosenheim and Rosen (1991) suggested that parasitoids preferentially host feed on small host and oviposit on larger host. *Anagyrus mangicola* host fed on crawlers and second-instar nymphs of *R. invadens* and did not oviposit in these individuals (Bokonon-Ganta et al. 1995). Although *Anagyrus* (= *Epidinocarsis*) *lopezi* (De Santis) host fed on all stages of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, the number of young nymphs fed upon was higher than that of the older mealybugs (Umeh 1988). We did not observe host feeding by *A. loecki* in this study. However, we occasionally observed *A. loecki* host feeding on young mealybugs in other laboratory and greenhouse experiments (Chong, personal observations).

Parasitoids, with foraging behavior influenced by natural selection, should lay clutches of eggs either to maximize fitness gained per host or fitness gained per unit time (Godfray 1994). Many studies suggest that clutch size should increase with host size (Godfray 1994, Jervis and Kidd 1996). The average clutch size of the gregarious *A. indicus* increased from 2.2 eggs per crawler to 11 eggs per adult mealybug (Nechols and Kikuchi 1985). Other studies on solitary mealybug parasitoids reported the same trend (Sagarra and Vincent 1999, Heng-Moss et al. 2001). *Anagyrus loecki* produced the largest clutch size (4 eggs per host) in third-instar female Madeira mealybugs. Even though adult mealybugs were the largest, they received a lower average clutch size of 2-3 eggs per host. The third-instar female nymphs represent a growing resource that would eventually achieve a larger size at adulthood and thus better quality hosts. On the other hand, adult female mealybugs represent hosts of maximum or declining quality due

to egg production. It is reasonable to suggest that *A. loecki* is able to discriminate between different host stages and to decide clutch size based on the future quality of the hosts and the fitness of the parasitoid progeny.

Most mealybug parasitoids were able to discriminate parasitized and unparasitized hosts (e.g. Boavida et al. 1995, Bokonon-Ganta et al. 1995, Cadee and van Alphen 1997, Islam and Copland 1997). Although solitary parasitoids were suggested to be more reluctant to superparasitize (Waage 1986), superparasitism was observed in some instances and contributed to clutch size larger than 1 egg per host. *Coccidoxenoides peregrinus* superparasitized even when there was an excess of hosts, causing the reported clutch size to exceed 1 (Ceballo and Walter 2004). The clutch size of *Anagyrus pseudococci* (Girault), another solitary species, also deviated from 1 because of superparasitism (Islam and Copland 1997). In Boavida et al (1995), superparasitism rates by *G. tebygi* were as high as 15% in crawlers and 33% in the second- and third-instar nymphs. The proportion of Madeira mealybugs superparasitized was higher for the third-instar and adult females (27-33%) than the crawlers and second-instar nymphs (8 and 14% respectively). This result suggests that the tendency of *A. loecki* to superparasitize increased with host size.

Superparasitism has significant consequences for the fitness of parasitoids in terms of development, survival, adult longevity and fecundity (Godfray 1994). Although there were tradeoffs between brood sizes and progeny fitness in terms of developmental time, the gregarious *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) produced larger broods in superparasitized *Pieris brassicae* (L.) (Lepidoptera: Pieridae) (Gu et al. 2003). In this study, the willingness of a female *A. loecki* to superparasitize older and larger mealybugs may be influenced by the higher quality of these hosts. Larger Madeira mealybugs may be able to support the development of

more parasitoids without decreasing the fitness of individual parasitoid. Unfortunately, we did not investigate the effects of superparasitism on the fitness of *A. loecki*.

Host stage suitability of *Anagyrus loecki*. The third-instar and pre-reproductive female Madeira mealybugs were the most suitable host stages for the development and survival of *A. loecki*. These two host stages were parasitized at a higher rates, supported high survival rates of the developing parasitoids, and produced the most numerous and the largest progeny with shorter duration of development. Studies on other mealybug parasitoids also suggested that larger hosts were more suitable than young nymphs for parasitoid development. Decreased developmental time in larger hosts appeared to be a common feature in parasitoid development (Nechols and Kikuchi 1985, Liu and Stanley 1996, Karamaouna and Copland 2000a, Neveu et al. 2000). Another gregarious encyrtid parasitoid, *Pseudophycus flavidulus* (Brèthes) De Santis, also completed development in a shorter time and produced a larger brood with a female-biased sex ratio in the larger individuals of *Pseudococcus viburni* (Signoret) (Karamaouna and Copland 2000a).

In idiobiont parasitoids, larger hosts are more profitable for a foraging parasitoid because these hosts contain a greater quantity of resources than smaller hosts, and thus are able to support the development of fitter progeny. Hosts attacked by koinobiont parasitoids continue to grow to a later stage and some are even able to reproduce before death (Cadee and van Alphen 1997). As a result, the relationship between the host body size and the fitness of the koinobiont parasitoid may not be as straightforward as that of the idiobiont parasitoid. Koinobiont parasitoids may suspend feeding and development in younger hosts until the hosts achieve an appropriate stage, thus with sufficient resources, to support the resumed development of the parasitoid larvae. Thus the developing parasitoids can achieve body size that would maximize their fitness (Harvey et al.

1994). A delay in development of parasitoids may be more pronounced in the first-instar parasitoid larvae (Smilowitz and Iwantsch 1975, Godfray 1994). Hormonal cues from the host stimulate the development and destructive feeding of the first-instar solitary koinobiont parasitoid *Hyposoter exiguae* (Hymenoptera: Ichneumonidae), which was suspended in young caterpillars (Smilowitz and Iwanstch 1975). As a result, the developmental rate of koinobiont parasitoids may be non-linear and reaches a plateau in larger hosts (Harvey et al. 1994).

Most life history studies on koinobiont parasitoids did not distinguish the host stage at which the parasitoid pupate and subsequently emerge, i.e. the host stage of mummification, from the host stage at the time of parasitism. In this study, we determined that the host stage at which mummification occurred had a significant influence on the fitness of *A. loecki*. Parasitoid larvae that allow young hosts to continue development and emerge from hosts mummified at a later developmental stage were more female-biased, more numerous and larger. Young larvae of *A. loecki* may have suspended development until the mealybugs reached a larger size to allow complete development of the parasitoids. The tendency to delay development by *A . loecki* was stronger when the brood size was larger because the quantity of resources required increase with brood size. The consequence of this delay was that the parasitoids deposited in younger nymphs and emerging from older hosts had similar fitness to parasitoids that completed their entire development in older hosts. We believe that for koinobiont parasitoids the host quality should be evaluated by the growth potential of a host as well as the host size at the time of parasitism. The difference in host stage at time of parasitism and host stage at the time of mummification can influence parasitoid development and should be studied in greater detail.

The mortality of eggs and young parasitoid larvae was not recorded in this study due to their small sizes and degradation after death. Dissections of mummies revealed low pupal

mortality rates in *A. loecki* that developed in older hosts. Parasitoid emergence rate was the lowest when the parasitoids were developing in ovipositing adult females, potentially due to the depletion of resources by mealybug egg production. Harvey et al. (2004) reported a similar non-linear or dome-shaped relationship between host size and parasitoid pre-adult mortality rate. The solitary koinobiont *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae) suffered from a higher mortality rate in the oldest and youngest age classes of the soybean looper, *Pseudoplusia includens* Walker (Lepidoptera: Noctuidae). Harvey et al. (2004) concluded that the older, larger hosts are not the best hosts and do not always produce progeny of highest fitness. Therefore conclusions based solely on the relationships between host size and progeny body size and/or developmental time may be misleading.

Under the model of host size–dependent sex allocation, more female parasitoids are expected to emerge from larger hosts, whereas more males emerge from smaller hosts (Charnov et al. 1981). This is because the relative gain in reproductive benefits from increased body size is greater in female progeny than in male progeny. Female-biased sex ratios in older hosts often reported in hymenopteran parasitoids (King 1993). The reproductive decisions by *A. loecki* observed in this study agreed with the host size-dependent (in this case host stage-dependent) sex allocation model: a higher proportion of female parasitoids with larger body size was produced from the third-instar nymphs and pre-reproductive adult mealybugs than the younger nymphs and ovipositing adults. Only 50% of the progeny that emerged from the ovipositing mealybugs were females. This result suggests that ovipositing females, with their reducing body content, were deemed less suitable for female progeny development by *A. loecki*. Studies on the gregarious *A. indicus* (Nechols and Kikuchi 1985) and *P. flavidulus* (Karamaouna and Copland 2000a), and the solitary *Aenasius vexans* Kerrich (Bertschy et al. 2000), *A. kamali* (Sagarra and

Vincent 1999) and *A. pseudococi* (Avidov et al 1967) also noted a proportion of males similar to that of *A. loecki*.

When the parasitoids emerged from hosts that had achieved further development since parasitism, the sex ratios were more female-biased compared to those that emerged from the original host stage of parasitism. The mealybugs that had survived and accrued body size at each molt became hosts of better nutritional quality to support development of a higher proportion of females. Crawlers that developed to adulthood produced nearly twice as many female parasitoids per mummy as individuals mummified in the second nymphal instar. There were 6 times as many female parasitoids emerged from adult mummies than from second-instar mummies if the mealybugs were parasitized at the second nymphal instar. Pre-reproductive female mealybugs that matured to egg production produced slightly more male parasitoids than young female mealybugs that were killed before resources were depleted by egg production.

Brood size was strongly influenced by host stage at the time of parasitism and host stage at the time of mummification. When considering only the host stage at the time of parasitism, the brood size of *A. loecki* was largest in the pre-reproductive mealybugs, followed by the third-instar nymphs. The gregarious *A. indicus* and *P. flavidulus* similarly produced the largest brood sizes in adult females (Nechols and Kikuchi 1985, Karamaouna and Copland 2000a, respectively). Mealybugs parasitized as first-instar nymphs continued to develop and those mummified at the second nymphal instar produced only 1 parasitoid per mummy. In contrast, crawlers in the same cohort that mummified as adults produced 3 parasitoids per mummy, which was similar to those emerged from mummies resulted from parasitism on pre-reproductive adult females. By suspending destructive feeding until the adult stage, the parasitoid larvae gained

additional host resources that could support the complete development of the majority if not all siblings in the same brood.

Comparisons between clutch sizes recovered in the preference experiments and brood sizes reported in the suitability study suggest the presence of pre-adult mortality. The greatest difference between clutch size (the maximum number of progeny assuming no mortality) and the brood size (the progeny number discounted by mortality) was observed in the third-instar mealybugs: the average brood size was 2.5 adults per host whereas the average clutch size was 4.2 eggs per host. Reasons proposed for low emergence rate in some host developmental stages include resource depletion, encapsulation and mortality in young nymphs (Van Driesche et al. 1987b, Islam and Copland 1997, Sagarra and Vincent 1999). The resource depletion hypothesis suggests that the mortality rate of parasitoids increases because resources available for parasitoid development decrease with host development and reproduction. We believe that the resource depletion hypothesis is not suitable in our study because the third-instar females continued to develop into adults thus representing an increasing, rather than declining, resource for the development of parasitoid larvae. Encapsulation is an important defense by the Coccoidea against their parasitoids (Blumberg 1997). Older mealybugs have been shown to be effective in encapsulating parasitoid eggs (Sagarra and Vincent 1999). No encapsulation of larvae or pupae of *A. loecki* was observed in this study. Eggs of *A. loecki* were not encapsulated in any host stage up to 24 h after deposition. However, encapsulation of parasitoid eggs or young larvae may occur more than 24 h after parasitism in third-instar nymphs. We did not examine the dissected mealybugs for encapsulation more than 24 h after parasitism. Also, we did not record the mortality rate in young nymphs due to ovipositor insertion in this study. As a result, we could not offer an explanation to the observed discrepancy between clutch size and brood size.

Results from this study suggest that increasing brood sizes by *A. loecki* might exacerbate the delay in parasitoid development and influence the progeny fitness by inducing competition among siblings. Positive correlations were found between brood size and developmental time of *A. loecki*, especially for parasitoids developing in the young nymphs. This result suggests that competition for host resource among the developing parasitoid larvae was related to the longer duration of development observed. Competition among siblings also caused the reduction of female and male body size (measured as hind tibial lengths) in third-instar nymphs and pre-reproductive mealybugs. Studies on different gregarious parasitoid species offered conflicting results. *Pseudaphycus flavidulus* produced progeny of the same size although brood size increased with mealybug body size (Karamaouna and Copland 2000a). The female parasitoids had the ability to allocate appropriate number of eggs to each host on the basis of the host size in order to produce progeny of similar fitness. However, Karamaouna and Copland (2000a) did not analyze for the direct correlation between brood size and progeny body size. Harvey (2000) reported that *C. glomerata* body size was negatively correlated with parasitoid brood size. The developmental time of *C. glomerata* was less affected by the brood size. The body size of *Metaphycus flavus* (Howard) and *Metaphycus stanleyi* Compere (Hymenoptera: Encyrtidae), both facultative gregarious parasitoids of soft scale insects (Hemiptera: Coccidae), either increased with or was unaffected by brood size (Bernal et al. 1999).

Implications for biological control programs. Two major parameters of concern in insectary rearing programs of biological control agents are female-biased sex ratio and large adult body size (Heinz 1998). Based on the results of this study, in order to achieve the goal of producing high quality female *A. loecki* for augmentative releases against the Madeira mealybug, the mass rearing system has to be designed so that a high proportion of the mealybug population consists

of third-instar nymphs and pre-reproductive adult females. These hosts supported a shorter developmental time and higher survival rate of *A. loecki.* The parasitoids also achieve larger size and a female-biased sex ratio when developing in these hosts. At a constant temperature of 25^{o}C, the completion of development from eggs to young adult females requires 30 d for the Madeira mealybug (Chong et al. 2003). The Madeira mealybug has a relatively long developmental period and it is tempting to shorten the production cycle of the parasitoids by initiating the colonies using smaller and younger hosts. However, such a production decision may produce smaller, mainly male parasitoids that are ineffective in providing control against the target pest either in the field or in the greenhouses.

It is also important to release *A. loecki* when the mealybug populations are mainly late-instar nymphs and adults. Although *A. loecki* parasitizes all stages of the Madeira mealybug, the parasitoids are more effective in searching and parasitizing hosts of later developmental stages. Since a higher proportion of females are produced from larger hosts, the chance of successful control and establishment will be greatly increased. However, the parasitized young adult females do continue to mature and eventually produce eggs. The effects of the parasitoid's koinobiont development on the hosts' survival and reproduction, and thus the host population dynamics, has to be studied to provide a more complete assessment of the potential of *A. loecki*.

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Table 3.1 List of behaviors used in for the behavioral observations.

Subjects	Behaviors	Characteristics
Parasitoid	Search	Walk with antennae drumming the leaf surface.
	Examine	Antennae continuously drumming a mealybug.
	Mount	Walk on top of a mealybug while continue antennal examination.
	Probe	Turn around and thrust ovipositor into the mealybug, often the wings are raised and body is rocking back and forth.
	Oviposition	Ovipositor inserted, with body and antennae remaining motionless or with slight shiver.
	Groom	Stop searching, and clean body, antennae and wings with legs.
	Rest	Stop all movement, body remains motionless.
	Host feeding	After retracting ovipositor, turn around and feed on host hemolymph exuded from puncture wound.
	Honeydew and water feeding	Feed on honeydew or water droplets on the leaf surface.
Mealybugs	Abdomen flipping	Violent twitching of body and repeated, rapid lifting of abdomen.
	Walk away	Retract stylet and walk away from original feeding site.
	Reflex bleeding	Exude drops of hemolymph through ostioles on head and abdomen.

Table 3.2 Mean duration (± SEM, in min) of behaviors of *Anagyrus loecki* when foraging on a coleus leaf disc with 10 Madeira mealybugs of different developmental stages. The behavior 'Feed' includes honeydew and water feeding, but not include host feeding.

Host stages	Behaviors						
	Search	Groom	Rest	Examine	Probe	Oviposit	Feed
				No-choice tests			
First-instar nymphs	0.48 ± 0.03a	0.24 ± 0.04ab	0.77	0.18 ± 0.02b	0.89 ± 0.06	6.33 ± 0.77b	0.81 ± 0.11
Second-instar nymphs	0.38 ± 0.03ab	0.24 ± 0.06ab	-	0.25 ± 0.02ab	0.89 ± 0.06	7.55 ± 0.52b	1.11 ± 0.14
Third-instar immature females	0.35 ± 0.03ab	0.54 ± 0.16a	2.64	0.24 ± 0.02ab	0.90 ± 0.06	15.47 ± 2.36a	0.91 ± 0.15
Third/fourth-instar immature males	0.27 ± 0.02b	0.07 ± 0.01c	3.89 ± 3.46	0.24 ± 0.03ab	0.88 ± 0.12	-	0.44 ± 0.09
Pre-ovipositing adult females	0.30 ± 0.03b	0.18 ± 0.03b	-	0.29 ± 0.03a	0.84 ± 0.05	9.78 ± 0.59b	1.16 ± 0.22
Ovipositing adult females	0.47 ± 0.04a	0.17 ± 0.02b	-	0.23 ± 0.01ab	1.01 ± 0.07	8.15 ± 0.63b	0.84 ± 0.16
				ANOVA *F* values			
	6.51 ****	3.70 **	0.14 NS	2.82 *	0.86 NS	9.29 ****	1.47 NS
				Choice tests			
No specific host stage identified	0.37 ± 0.05	0.25 ± 0.06	2.37 ± 0.98				0.88 ± 0.16
First-instar nymphs				0.10 ± 0.02b	0.85 ± 0.13	4.91 ± 2.00	
Second-instar nymphs				0.15 ± 0.03b	0.87 ± 0.15	8.35 ± 2.06	
Third-instar immature females				0.19 ± 0.03b	0.90 ± 0.09	15.65 ± 2.31	
Pre-ovipositing adult females				0.31 ± 0.04a	0.75 ± 0.07	10.83 ± 2.20	
Ovipositing adult females				0.32 ± 0.08a	0.93 ± 0.13	7.89 ± 1.62	
				ANOVA *F* values			
	-	-	-	3.45 *	0.61 NS	2.25 NS	-

****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA; SAS, 1985).

Table 3.3 Mean (± SEM) proportion of Madeira mealybugs antennally examined, probed and oviposited by *Anagyrus loecki* in no choice and choice tests.

	Host stages						ANOVA
	First-instar nymphs (Crawlers)	Second-instar nymphs	Third-instar immature females	Third/fourth-instar immature males	Pre-ovipositing adult female	Ovipositing adult females	**F**
				No-choice tests			
Total number of available hosts per arena	10	10	10	10	10	10	
Proportion of hosts examined	0.57 ± 0.04a	0.58 ± 0.04a	0.43 ± 0.04a	0.20 ± 0.05b	060 ± 0.04a	0.58 ± 0.04a	3.03 *
Proportion of examined hosts probed	0.84 ± 0.04a	0.87 ± 0.03a	0.75 ± 0.04ab	0.31 ± 0.10c	0.65 ± 0.05ab	0.56 ± 0.05b	8.70 ****
Proportion of probed hosts oviposited	0.59 ± 0.07a	0.71 ± 0.06a	0.79 ± 0.06a	0b	0.82 ± 0.04a	0.80 ± 0.06a	9.99 ****
Proportion of examined hosts oviposited	0.75 ± 0.05a	0.75 ± 0.05a	0.71 ± 0.05a	0b	0.60 ± 0.04a	0.52 ± 0.05a	5.87****
Proportion of total available hosts oviposited	0.28 ± 0.04a	0.35 ± 0.03a	0.24 ± 0.03a	0b	0.30 ± 0.02a	0.25 ± 0.03a	9.86 ****
				Choice tests			
Total number of available hosts per arena	2	2	2	0	2	2	
Proportion of hosts examined	0.20 ± 0.06c	0.35 ± 0.06bc	0.55 ± 0.09ab	-	0.75 ± 0.09a	0.53 ± 0.08ab	8.81 ****
Proportion of examined hosts probed	0.38 ± 0.18ab	0.27 ± 0.12b	0.60 ± 0.12ab	-	0.88 ± 0.05a	0.50 ± 0.11ab	4.15 **
Proportion of probed hosts oviposited	0.67 ± 0.33	1.0	0.90 ± 0.27	-	0.75 ± 0.10	0.75 ± 0.13	0.69 NS
Proportion of examined hosts oviposited	0.38 ± 0.18b	0.27 ± 0.12b	0.60 ± 0.12ab	-	0.74 ± 0.09a	0.47 ± 0.11ab	3.26*
Proportion of total available hosts oviposited	0.05 ± 0.03b	0.10 ± 0.05b	0.30 ± 0.08ab	-	0.48 ± 0.08a	0.20 ± 0.06b	7.31 ****

****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA; SAS, 1985).

Table 3.4 Mean (± SEM) developmental time (in days) of *Anagyrus loecki* emerged from various developmental stages of the Madeira mealybugs.

Exposed host stages	Combined	Mummified host stages				ANOVA *F*
		Second-instar nymphs	Third-instar immature females	Pre-ovipositing adult females	Ovipositing adult females	
First-instar nymphs	27.6 ± 0.3A	26.6 ± 4.7b	29.8 ± 3.1a	34.0 ± 6.6a	-	13.74 ****
Second-instar nymphs	19.8 ± 0.3B	16.8 ± 2.0c	20.4 ± 3.6b	24.6 ± 4.2a	-	122.94 ****
Third-instar immature females	20.6 ± 0.3B	-	19.8 ± 3.7b	21.0 ± 4.3a	-	4.24 *
Pre-ovipositing adult females	16.2 ± 0.1C	-	-	16.1 ± 1.3	16.3 ± 2.1	1.70 NS
Ovipositing adult female	15.6 ± 0.1C	-	-	-	16.0 ± 0.7	
ANOVA *F*	283.11 ****					

****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA; PROC GLM, SAS, 1985).
Means of developmental time within combined host stages with the same capital letters are not significantly different among the exposed host stages. Means of developmental time within each exposed host stages with the same small letters are not significantly different among the mummified host stages (Tukey's HSD, $\alpha = 0.05$; PROC GLM, SAS, 1985).

Table 3.5 Mean (± SEM) brood sizes of *Anagyrus loecki* emerged from various developmental stages of the Madeira mealybugs.

Exposed host stages	Combined	Mummified host stages				ANOVA *F*
		Second-instar nymphs	Third-instar immature females	Pre-ovipositing adult females	Ovipositing adult females	
First-instar nymphs	1.2 ± 0.1D	1.1 ± 0.2c	1.6 ± 0.9b	2.7 ± 0.6a	-	45.93 ****
Second-instar nymphs	1.6 ± 0.1C	1.0 ± 0.1b	1.2 ± 0.4b	3.2 ± 1.3a	-	238.53 ****
Third-instar immature females	2.5 ± 0.1B	-	1.3 ± 0.5b	3.0 ± 1.3a	-	120.48 ****
Pre-ovipositing adult females	2.9 ± 0.1A	-	-	3.0 ± 1.8	2.9 ± 1.6	0.02 NS
Ovipositing adult female	2.0 ± 0.1C	-	-	-	2.0 ± 0.1	
ANOVA *F*	65.42 ****					

****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA; PROC GLM, SAS, 1985).

Means of brood sizes within combined host stages with the same capital letters are not significantly different among the exposed host stages. Means of brood sizes within each exposed host stages with the same small letters are not significantly different among the mummified host stages (Tukey's HSD, $\alpha = 0.05$; PROC GLM, SAS, 1985).

Table 3.6 Mean (± SEM) proportion males of *Anagyrus loecki* emerged from various developmental stages of the Madeira mealybugs.

Exposed host stages	Combined	Mummified host stages				ANOVA *F*
		Second-instar nymphs	Third-instar immature females	Pre-ovipositing adult females	Ovipositing adult females	
First-instar nymphs	0.57 ± 0.03A	0.82 ± 0.07a	0.62 ± 0.09b	0.51 ± 0.11b	-	3.38 *
Second-instar nymphs	0.42 ± 0.03B	0.89 ± 0.07a	0.22 ± 0.06b	0.27 ± 0.03b	-	69.71 ****
Third-instar immature females	0.34 ± 0.02C	-	0.36 ± 0.02a	0.32 ± 0.02b	-	24.93 ****
Pre-ovipositing adult females	0.36 ± 0.02C	-	-	0.35 ± 0.04	0.37 ± 0.03	0.31 NS
Ovipositing adult female	0.50 ± 0.05AB	-	-	-	0.50 ± 0.05	
ANOVA *F*	23.84 ****					

****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA; PROC GLM, SAS, 1985).
Means of proportion males within combined host stages with the same capital letters are not significantly different among the exposed host stages. Means of proportion males within each exposed host stages with the same small letters are not significantly different among the mummified host stages (Tukey's HSD, $\alpha = 0.05$; PROC GLM, SAS, 1985).

Table 3.7 Mean (± SEM) female and male hind tibial length of *Anagyrus loecki* emerged from various developmental stages of the Madeira mealybugs.

Exposed host stages	Combined	Mummified host stages				ANOVA *F*
		Second-instar nymphs	Third-instar immature females	Pre-ovipositing adult females	Ovipositing adult females	
			Females			
First-instar nymphs	0.33 ± 0.01B	0.32 ± 0.01b	0.35 ± 0.01a	0.34 ± 0.02ab	-	12.15 ****
Second-instar nymphs	0.34 ± 0.01AB	0.29 ± 0.01c	0.35 ± 0.01a	0.34 ± 0.01b	-	51.87 ****
Third-instar immature females	0.35 ± 0.01A	-	0.35 ± 0.01	0.35 ± 0.01	-	0.97 NS
Pre-ovipositing adult females	0.35 ± 0.01A	-	-	0.36 ± 0.01	0.35 ± 0.01	1.29 NS
Ovipositing adult female	0.34 ± 0.01AB	-	-	-	0.34 ± 0.01	
ANOVA *F*	12.20 ****					
			Males			
First-instar nymphs	0.27 ± 0.01C	0.26 ± 0.01b	0.28 ± 0.01a	0.24 ± 0.01b	-	8.69 ***
Second-instar nymphs	0.27 ± 0.01C	0.26 ± 0.01b	0.29 ± 0.01a	0.28 ± 0.01a	-	31.45 ****
Third-instar immature females	0.29 ± 0.01B	-	0.29 ± 0.01	0.29 ± 0.01	-	1.26 NS
Pre-ovipositing adult females	0.30 ± 0.01A	-	-	0.30 ± 0.01	0.30 ± 0.01	0.23 NS
Ovipositing adult female	0.29 ± 0.01B	-	-	-	0.29 ± 0.01	
ANOVA *F*	30.72 ****					

****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P \leq 0.05$; NS, $P > 0.05$ (ANOVA; PROC GLM, SAS, 1985).

Means of proportion males within combined host stages with the same capital letters are not significantly different among the exposed host stages. Means of proportion males within each exposed host stages with the same small letters are not significantly different among the mummified host stages (Tukey's HSD, $\alpha = 0.05$; PROC GLM, SAS, 1985).

Table 3.8 Spearman's correlation coefficients and *p*-values for the relationships between sibling numbers and hind tibial lengths of female and male *Anagyrus loecki* emerged from the Madeira mealybugs parasitized at various developmental stages.

Exposed host stages	Spearman's correlation coefficients	*P*-values
	Females	
First-instar nymphs	0.2246	0.0295
Second-instar nymphs	-0.0825	0.1823
Third-instar immature females	-0.3832	< 0.0001
Pre-ovipositing adult females	-0.6516	< 0.0001
Ovipositing adult females	-0.0152	0.9045
	Males	
First-instar nymphs	-0.0447	0.5964
Second-instar nymphs	0.3908	< 0.0001
Third-instar immature females	-0.3697	< 0.0001
Pre-ovipositing adult females	-0.5847	< 0.0001
Ovipositing adult females	-0.0681	0.5841

Figure Legends

Fig. 3.1. Observed sequence of oviposition behavior of *Anagyrus loecki* in no-choice tests. The mealybug development stages were crawlers (A), second-instar nymphs (B), third-instar immature females (C), third- or fourth-instar immature males (D), pre-ovipositing adult females (E), and ovipositing adult females (F). The number above each line represents the proportion of subsequent events. Proportions of events not accounted for in this figure were attributed to grooming, resting, and feeding.

Fig. 3.2. Total duration of observation (in min) per *Anagyrus loecki* foraging for mealybugs of various developmental stages in the no-choice (A) and choice (B) tests. The host stages were crawlers (N1), second-instar nymphs (N2), third-instar immature females (N3F), third- or fourth instar immature males (N3M), pre-ovipositing adult females (Pre-ovip), and ovipositing adult females (Ovip).

Fig. 3.3. Encounter rates of *Anagyrus loecki* with Madeira mealybugs of various developmental stages in host stage preference study. The host stages were crawlers (N1), second-instar nymphs (N2), third-instar immature females (N3F), third- or fourth instar immature males (N3M), pre-ovipositing adult females (Pre-ovip), and ovipositing adult females (Ovip). Bars annotated with the same letters were not significantly different.

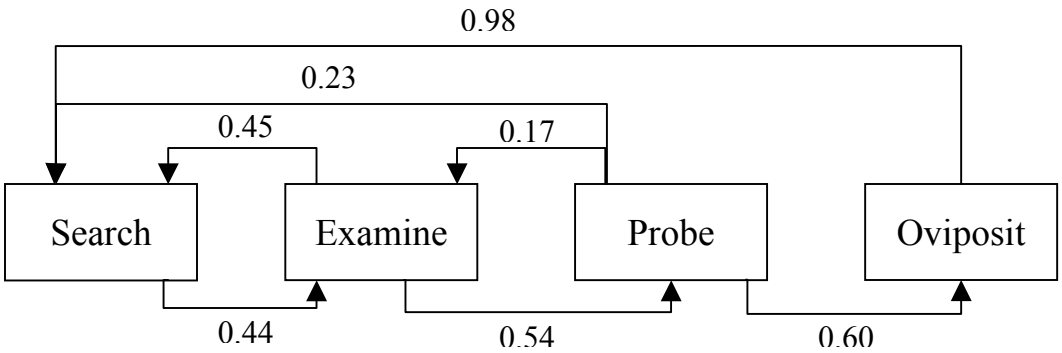
Fig. 3.4. Means (± SEM) of clutch sizes of *Anagyrus loecki* recovered from Madeira mealybugs of various developmental stages. The host stages were crawlers (N1), second-instar nymphs (N2), third-instar immature females (N3F), pre-ovipositing adult females (Pre-ovip), and ovipositing adult females (Ovip).Bars annotated with the same letters were not significantly different.

Fig. 3.5. Proportions of total available mealybugs parasitized by *Anagyrus loecki* in the no-choice (A) and choice (B) tests of the host stage suitability study. The host stages were crawlers (N1), second-instar nymphs (N2), third-instar immature females (N3F), pre-ovipositing adult females (Pre-ovip), and ovipositing adult females (Ovip). Bars topped with the same letters were not significantly different.

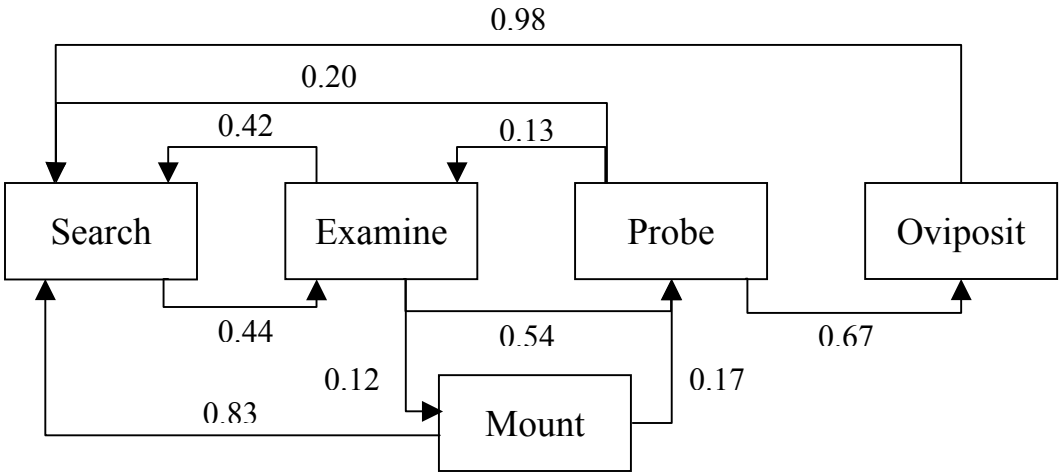
Fig. 3.6. Mean progeny emergence rates of *Anagyrus loecki* from Madeira mealybugs of various developmental stages in the no-choice (A) and choice (B) tests. The host stages were crawlers (N1), second-instar nymphs (N2), third-instar immature females (N3F), pre-ovipositing adult females (Pre-ovip), and ovipositing adult females (Ovip). Bars with the same letters imbedded were not significantly different.

Fig. 3.7. Proportions of mummified host stages from the various parasitized host stages of the Madeira mealybugs: crawlers (N1), second-instar nymphs (N2), third-instar immature females (N3F), pre-ovipositing adult females (Pre-ovip), and ovipositing adult females (Ovip).

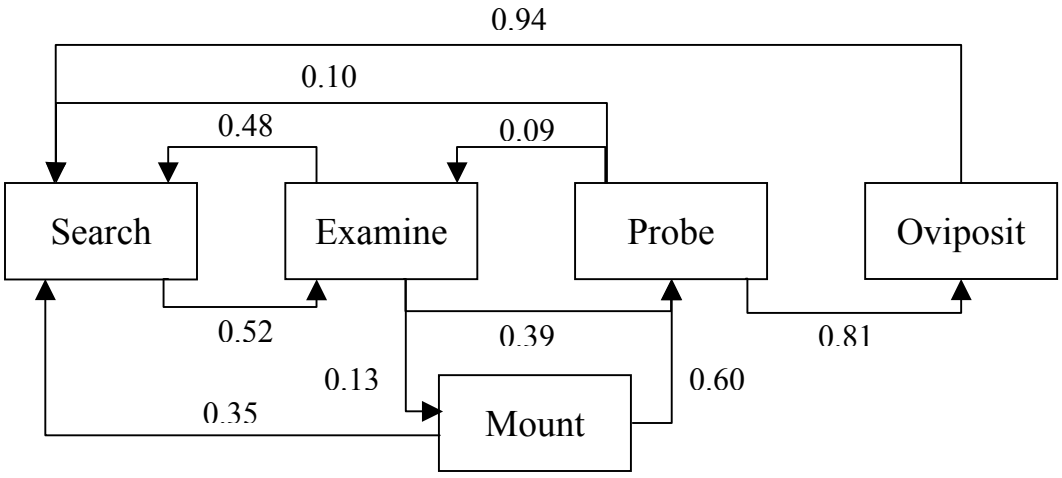
A


0.98
0.23
0.45
0.17
Search
Examine
Probe
Oviposit
0.44
0.54
0.60

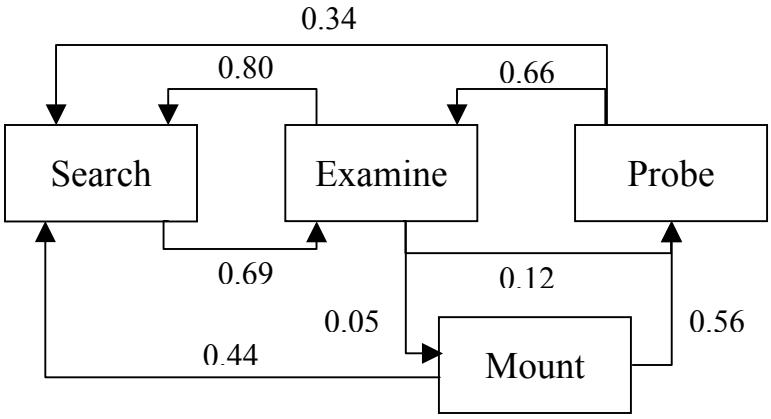
B


0.98
0.20
0.42
0.13
Search
Examine
Probe
Oviposit
0.44
0.54
0.67
0.12
0.17
0.83
Mount

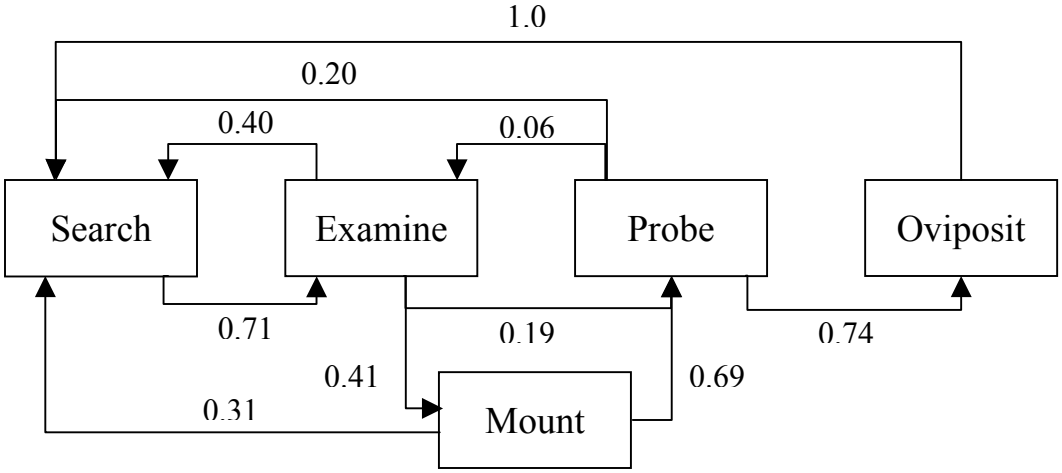
C


0.94
0.10
0.48
0.09
Search
Examine
Probe
Oviposit
0.52
0.39
0.81
0.13
0.60
0.35
Mount

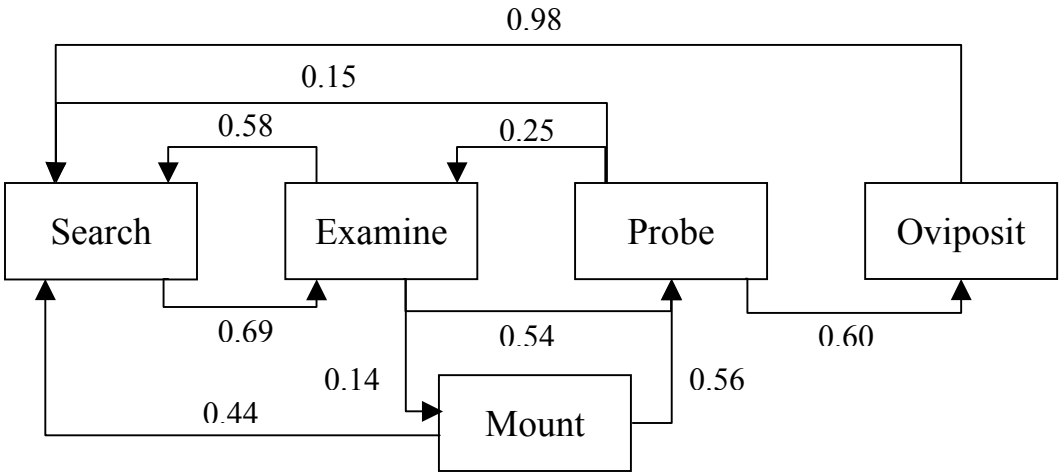
D


0.34
0.80
0.66
Search
Examine
Probe
0.69
0.12
0.05
0.56
0.44
Mount

E


1.0
0.20
0.40
0.06
Search
Examine
Probe
Oviposit
0.71
0.19
0.74
0.41
0.69
0.31
Mount

F


0.98
0.15
0.58
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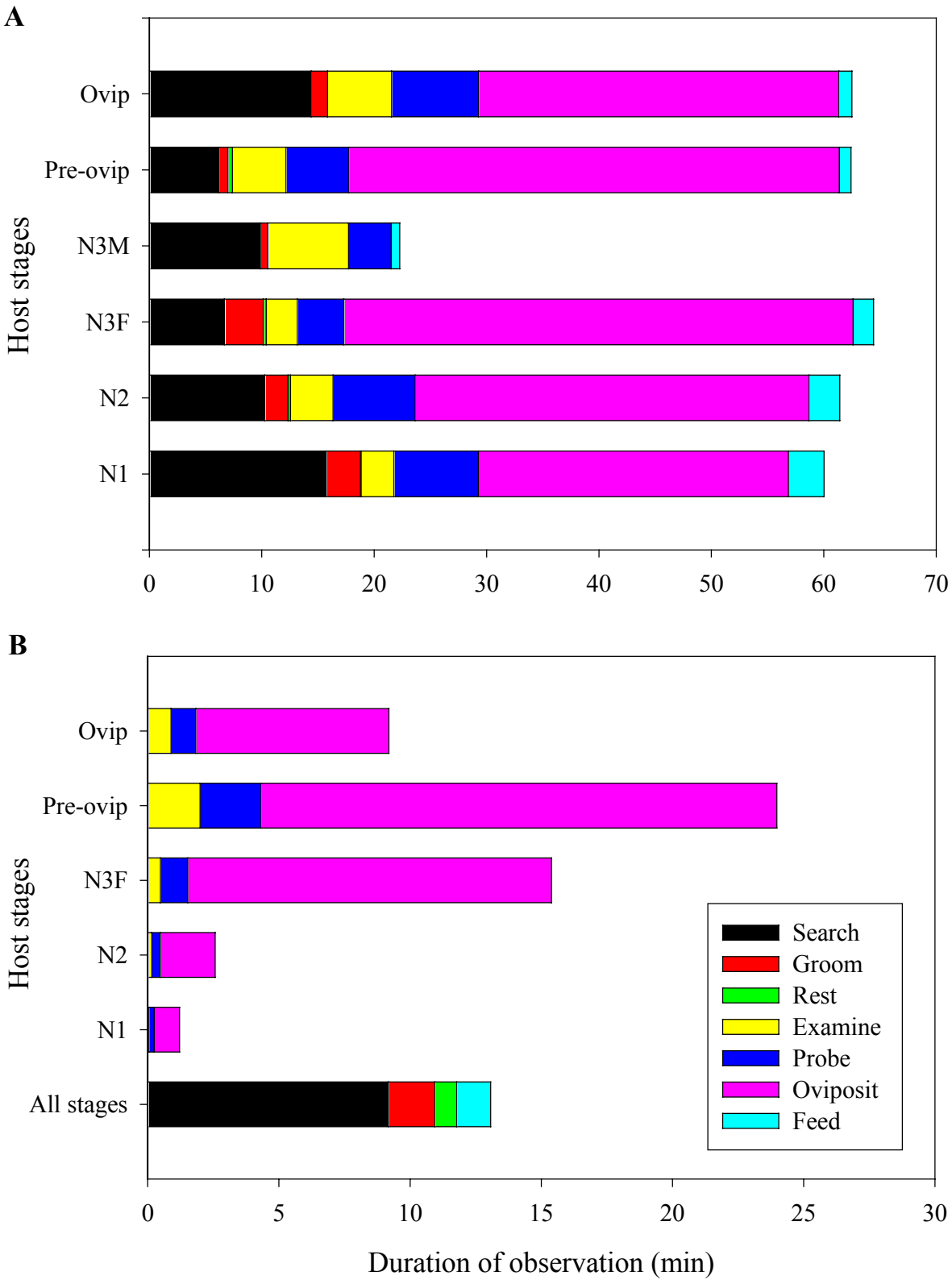
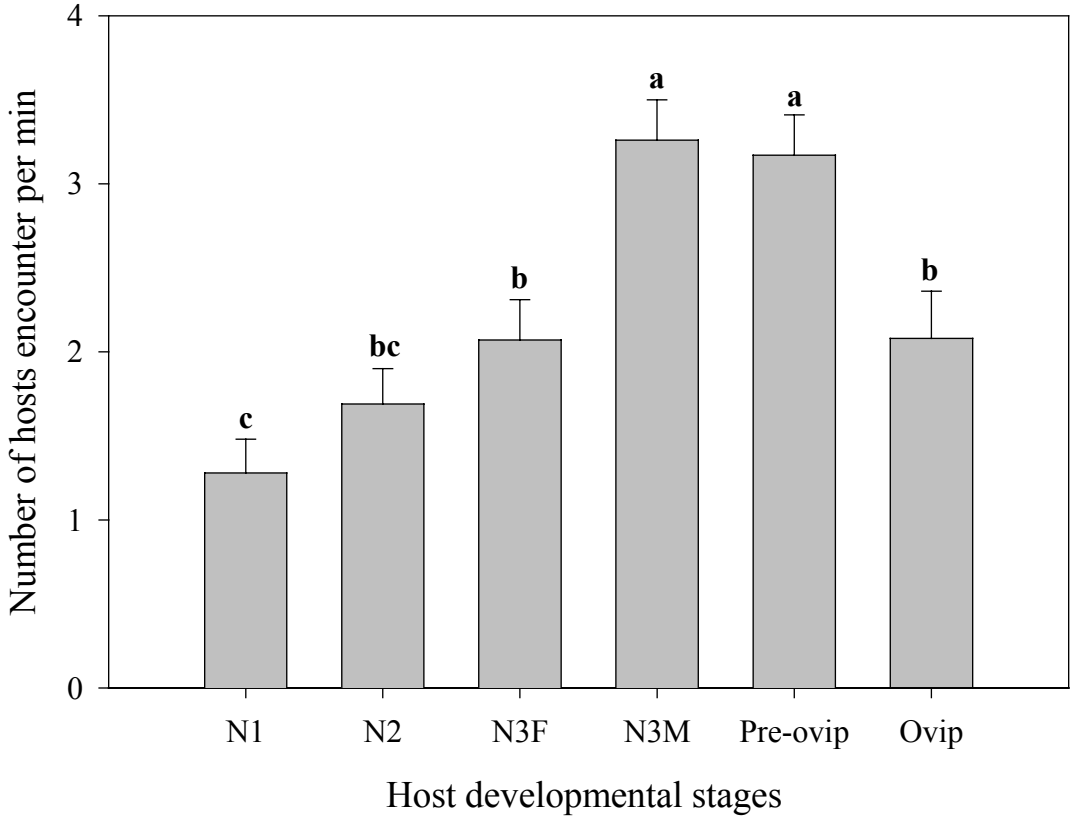
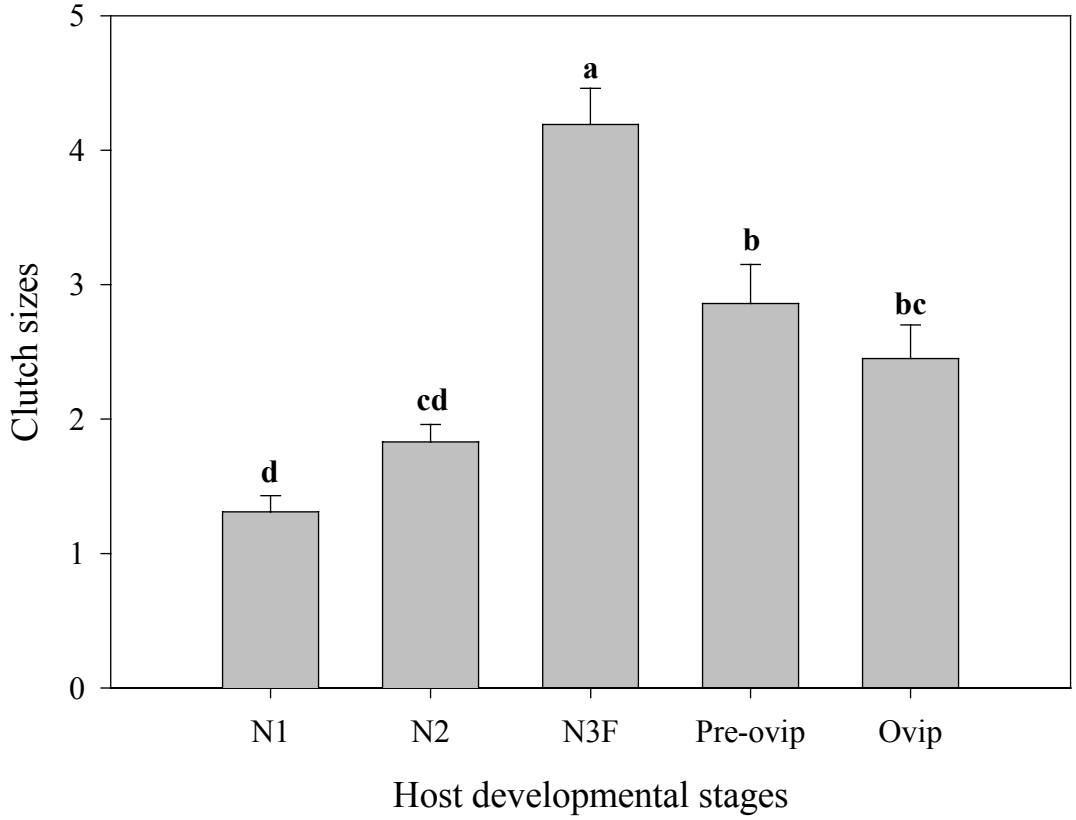
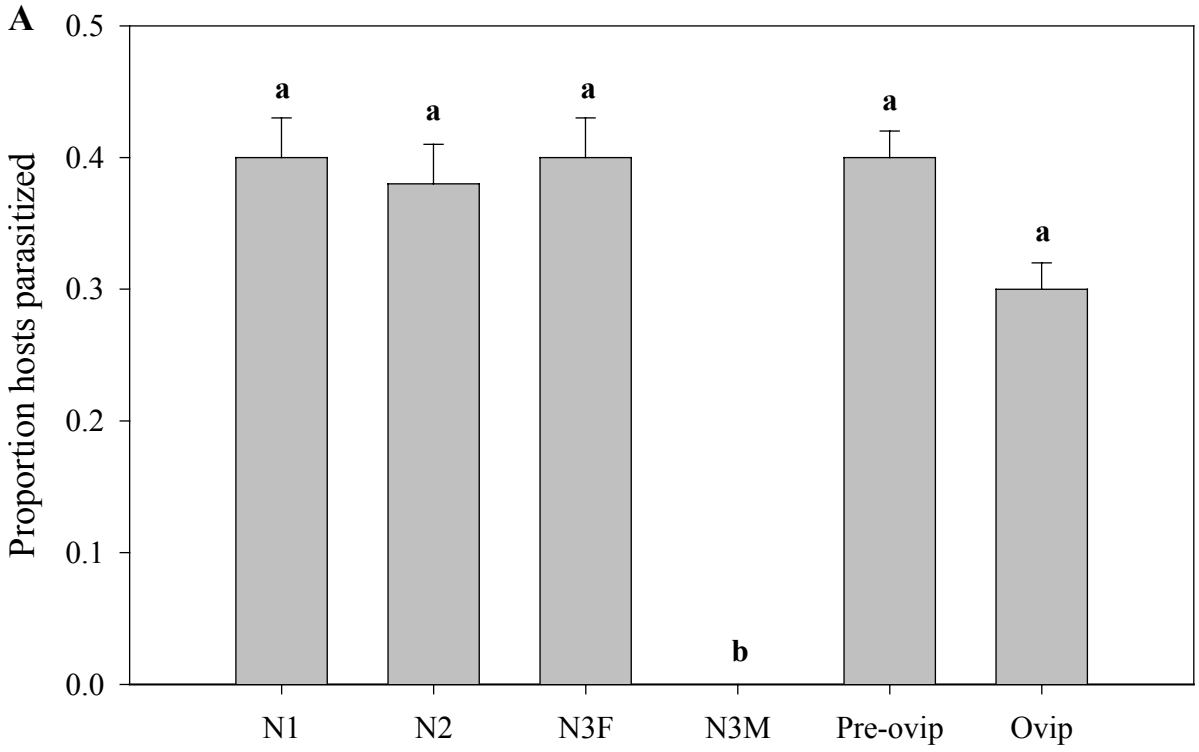
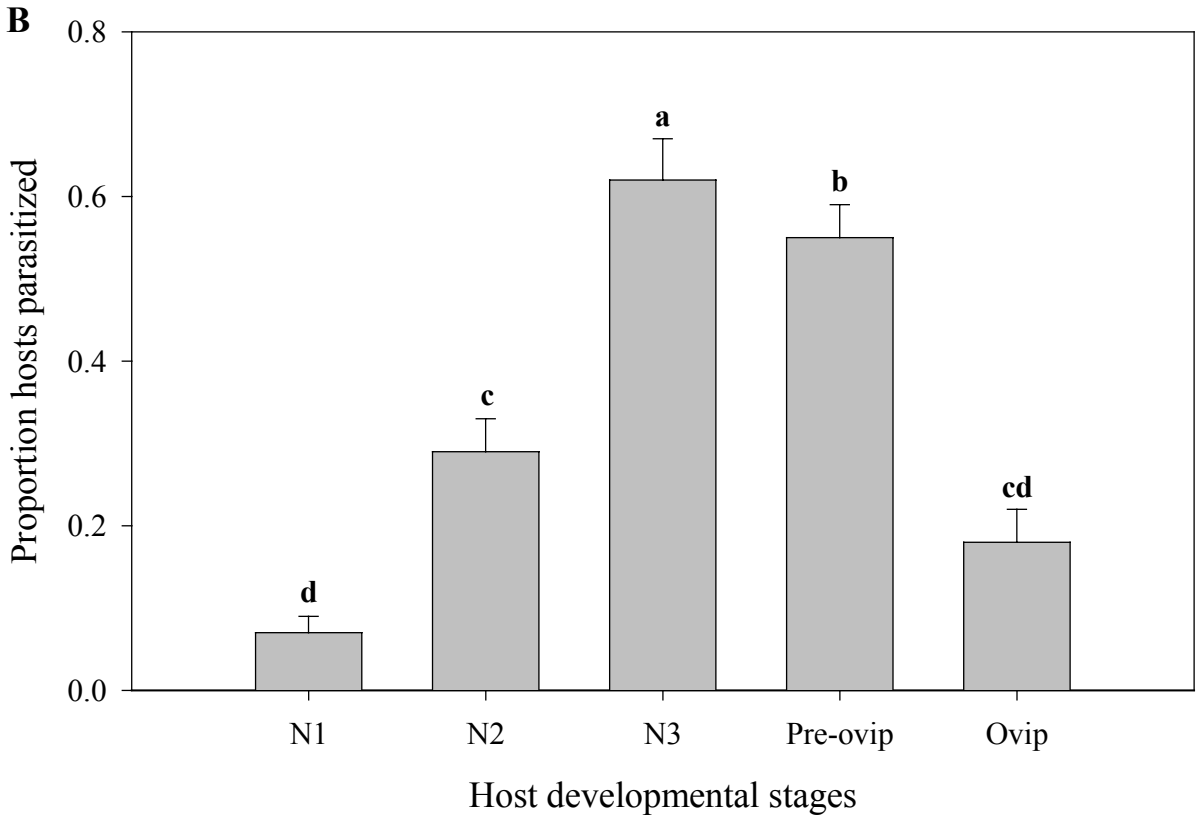
Search
Examine
Probe
Oviposit
0.69
0.54
0.60
0.14
0.56
0.44
Mount

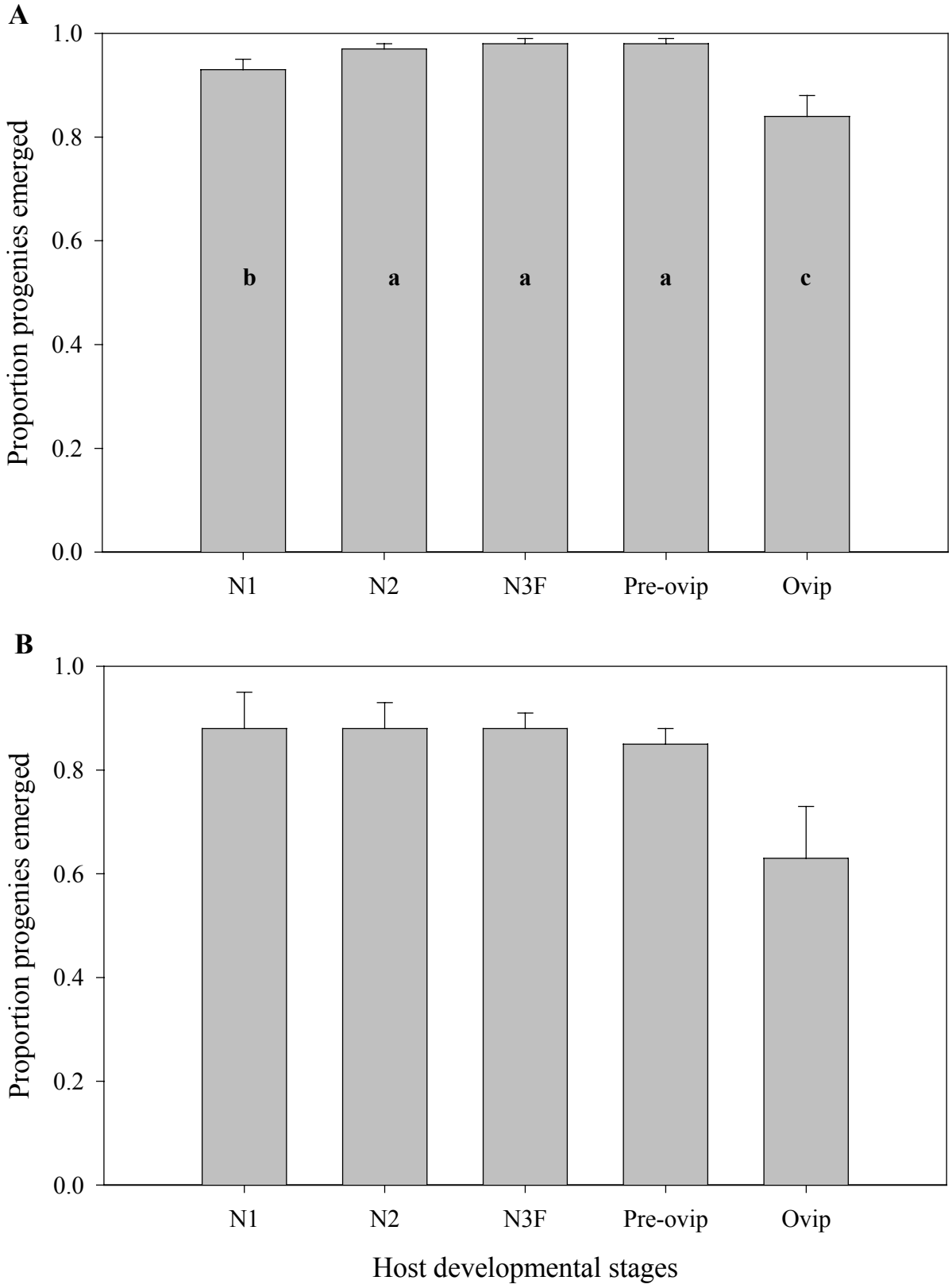

A
Ovip
Pre-ovip
N3M
N3F
N2
N1
Host stages
0
10
20
30
40
50
60
70
B
Ovip
Pre-ovip
N3F
N2
N1
All stages
Host stages
Search
Groom
Rest
Examine
Probe
Oviposit
Feed
0
5
10
15
20
25
30
Duration of observation (min)

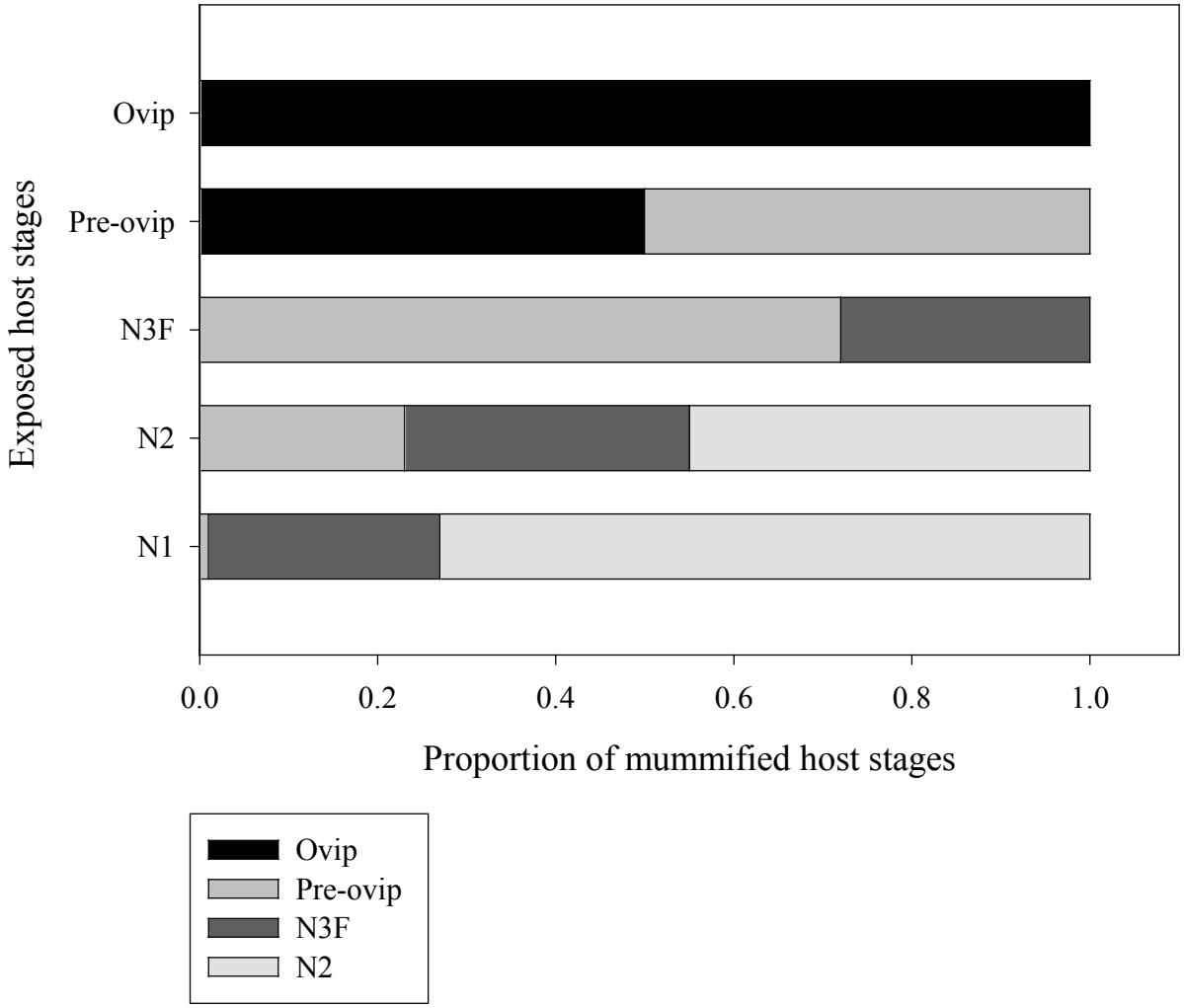

Number of hosts encounter per min
0
1
2
3
4
c
bc
b
a
a
b
N1
N2
N3F
N3M
Pre-ovip
Ovip
Host developmental stages


5
4
3
2
1
0
Clutch sizes
a
b
bc
cd
d
N1
N2
N3F
Pre-ovip
Ovip
Host developmental stages


A
Proportion hosts parasitized
0.0
0.1
0.2
0.3
0.4
0.5
a
a
a
b
a
a
N1
N2
N3F
N3M
Pre-ovip
Ovip


B
Proportion hosts parasitized
0.0
0.2
0.4
0.6
0.8
d
c
a
b
cd
N1
N2
N3
Pre-ovip
Ovip
Host developmental stages


A
Proportion progenies emerged
1.0
0.8
0.6
0.4
0.2
0.0
b
a
a
a
c
N1
N2
N3F
Pre-ovip
Ovip
B
Proportion progenies emerged
1.0
0.8
0.6
0.4
0.2
0.0
N1
N2
N3F
Pre-ovip
Ovip
Host developmental stages


Exposed host stages
Ovip
Pre-ovip
N3F
N2
N1
0.0
0.2
0.4
0.6
0.8
1.0
Proportion of mummified host stages
Ovip
Pre-ovip
N3F
N2

CHAPTER 4

FUNCTIONAL RESPONSE OF THE MEALYBUG PARASITOID *ANAGYRUS LOECKI* (HYMENOPTERA: ENCYRTIDAE)[1]

[1]Chong, J.-H., and R. D. Oetting. 2005. To be submitted to the Annals of the Entomological Society of America.

ABSTRACT The effect of host density on functional response and reproduction of *Anagyrus loecki* Noyes & Menezes, a parasitoid of the Madeira mealybug (*Phenacoccus madeirensis* Green), was determined in laboratory conditions. Pre-reproductive adult female mealybugs were exposed for 24 h to a single naïve parasitoid at the host densities of 2, 5, 10, 20, 30, 40 and 50 mealybugs per arena. An exponential decrease in the proportion of parasitized hosts with increasing host density suggested a type II functional response. The instantaneous attack rate (*a*) was estimated at 0.03 and the handling time (T_h) was estimated at 1 h. Emergence rate, progeny number, brood size and sex ratio were significantly influenced by host density. The proportion of progeny that successfully emerged was lowest at the lowest host density, and increased to above 95% among the higher host densities. Individual female *A. loecki* produced 6 offspring at 2 mealybugs per arena and 31 progeny at 50 mealybugs per arena, showing a significant trend of increasingly higher progeny numbers at higher host densities. Brood size decreased with increased host density from 3 parasitoids per mummy at 2 mealybugs per arena to 1.8 parasitoids per mummy at 50 mealybugs per arena. The proportion of males was lowest with 2 mealybugs per arena, and averaged 0.29-0.33 between the host densities of 2-50 mealybugs per arena.

KEY WORDS *Anagyrus loecki*, *Phenacoccus madeirensis*, functional response, numerical response, biological control

Anagyrus loecki Noyes & Menezes is a parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), a common pest of greenhouse ornamental production in the Southeastern United States. The natural distribution of this parasitoid species includes Costa Rica, Saint Kitts, Florida and Texas (Noyes 2000). *Anagyrus loecki* attacks several economically important mealybug species including the Madeira mealybug, the papaya mealybug (*Paracoccus marginatus* Williams and Granada de Willink) and the mealybug *Dysmicoccus* nr. *hurdi* (Noyes 2000). The release of an encyrtid parasitoid complex, including *A. loecki*, in the Dominican Republic achieved successful control of the papaya mealybug (D. Meyerdirk, personal communication).

The ability of a natural enemy to regulate pest populations is generally believed to be dependent on the predator's functional and numerical responses (Solomon 1949). The functional response of a parasitoid describes the relationship between the number of hosts parasitized and the host density. The numerical response is defined as the change in the parasitoid's reproductive output at varying host density. Parasitoids that exhibit a type I functional response search for hosts randomly within a patch and attack hosts at a constant rate, resulting in a linear relationship between the number of parasitized host and the host density. The proportion of hosts parasitized by a parasitoid with a type II functional response decreases exponentially as the host density increases. The type III functional response is described by an initial increase and subsequent decrease in the proportion of hosts parasitized as the host density increases. Changes in the proportion of parasitized host are often limited by available searching time or satiation in predators (Holling 1959, Mills 1982), and egg depletion or handling time in parasitoids (Getz and Mills 1996).

Functional response analyses are often used to predict the dynamics of host-parasitoid interactions and the potential of a natural enemy to regulate pest populations (Oaten and Murdoch 1975). A type II functional response with a decelerating parasitism rate has the potential to destabilize host-parasitoid population dynamics due to an inverse density-dependent mortality of the hosts (Hassell 1978). In contrast, type III functional response, which incorporates density-dependent host mortality, may stabilize the dynamics (Murdoch and Oaten 1975). A natural enemy that exhibits a density-dependent functional response is believed to be more effective (Solomon 1949).

The Madeira mealybug has caused an estimated $ 71 million annually in losses to the ornamental production and maintenance in Georgia (Oetting et al. 2002). Due to the increasing difficulty in managing the Madeira mealybug, biological control is currently being evaluated as part of integrated pest management program. As a part of an evaluation of *A. loecki* as a biological control agent of the Madeira mealybug, the goals of this study were to describe functional response of *A. loecki* and to assess the effects of varying host density on the reproduction of the parasitoid.

Materials and Methods

Preparation of parasitoids and mealybugs. The Madeira mealybugs used in this study were collected from a greenhouse colony maintained on coleus (*Solenostemon scutellarioides* Thonn., var. 'Park's Brilliance Mix') at the University of Georgia, Griffin Campus, Griffin, GA. To obtain pre-reproductive adult female mealybugs for this study, each potted coleus was infested with numerous Madeira mealybug ovisacs, which were collected from an insectary colony maintained on sprouted russet potatoes (*Solanum tuberosum* L.) at the Griffin Campus, 1 mo before the commencement of the study. To standardize the quality of the hosts, adult Madeira

mealybugs were visually graded and only individuals of similar sizes (3-4 mm) were collected. The mealybugs were transferred into their respective experimental arenas 16 h prior to the experiment to allow settlement and feeding on the coleus leaves.

Laboratory colonies of *A. loecki* were established with parasitoids received from the University of Florida, Mid-Florida Research and Education Center, Apopka, FL in September 2000. The parasitoids used in this study were reared in laboratory cultures on the Madeira mealybugs maintained on sprouted russet potatoes. Mummies were collected from the laboratory colonies, isolated individually in gelatin capsules (no. 1, Eli Lilly and Co., Indianapolis, IN), and held in an environmental chamber (model I-35VL, Percival Manufacturing Co., Boone, IA) maintained at 25 ^{o}C until parasitoid emergence. To ensure successful mating and egg maturation, parasitoids of both sexes, which had emerged within 24 h, were collected. These parasitoids were held in a group for 48 h prior to the experiment at 25^{o}C in plastic vials (70 by 25 mm) supplied with streaks of 50% honey solution. Only female parasitoids of similar body length (1.7-2.0 mm) were selected for this study to limit the potential size-based difference in attack efficiency among the parasitoids.

Experimental Procedures. The experimental arena consisted of an excised coleus leaf with its petiole inserted through a hole in the bottom of a Petri dish (100 by 20 mm) and into a cup of water. Streaks of diluted honey were provided as water and carbohydrate sources for the parasitoids in the Petri dishes. All experimental arenas were held in an environmental chamber maintained at 25 ^{o}C, 90±3% RH, and 16 h photoperiod.

A single 48-h-old female *A. loecki* was introduced into an arena containing mealybugs at one of the seven densities: 2, 5, 10, 20, 30, 40 and 50 mealybugs per arena. The parasitoid was allowed to forage in the arena for 24 h. The arena was covered with chiffon to allow ventilation

and prevent escape of the insects. After the removal of the parasitoids, the mealybug cohorts were incubated in the environmental chamber and examined for mummies every 5 d. Mummies were collected and isolated in individual gelatin capsules, and incubated until parasitoid emergence. The numbers of mummy were recorded for each host density. Adult parasitoids were counted and sexed upon emergence. Brood size, i.e., the number of parasitoids emerged from each mummy, was determined. All mummies were dissected at the end of the experiment to verify the successful parasitoid emergence. Each host density treatment was replicated 40 times.

Data analysis. Following the model by Nicholson and Bailey (1935), the type I functional response model is described as

$N_a = aTN_o$.

Type II functional response is often referred to as the 'Holling's disk equation' (Holling 1959):

$$N_a = \frac{aTN_o}{1 + aT_hN_o}.$$

Hassell (1978) provided a model for the type III functional response:

$$N_a = \frac{dN_oT + bN_o^2T}{a - cN_o + dN_oT + bN_o^2T_h}.$$

In these models, N_a is the number of mealybugs parasitized, N_o is the initial host density, T is the time available for searching and parasitism, *a* is the instantaneous attack rate, the parameters *b*, *c* and *d* are constants related to attack rate, and T_h is the handling time. The searching time (T) was set at 24 h, the total duration of the experiment. Although the above functional response models do not incorporate host depletion (Juliano 2001), we believed that the high rate of self-superparasitism observed in *A. loecki* (Chong and Oetting, Chapter 3) reduced the effects of host depletion and thus justified the use of these models.

A logistic regression analysis for maximum likelihood (PROC CATMOD; SAS Institute 1999) was performed to fit the data to the logistic model

$$\frac{N_a}{N_o} = \frac{\exp(P_0 + P_1 N_o + P_2 N_o^{\,2} + P_3 N_o^{\,3})}{1 + \exp(P_0 + P_1 N_o + P_2 N_o^{\,2} + P_3 N_o^{\,3})}$$

to determine if the proportion of parasitized hosts fit the patterns predicted by the three functional response models (Juliano 2001). The parameters N_o is the host density, N_a is the number of mealybugs parasitized, and P_0, P_1, P_2 and P_3 are the logistic regression parameters related to the slope of the curve. The slope of the proportion of parasitized hosts near the lowest host density predicted by the type I response is zero (i.e. P_0 is zero), that predicted by the type II functional response is declining (i.e., P_0 is negative), and that by the type III functional response is accelerating (i.e., P_0 is positive and P_1 is negative). The numbers of mealybugs parasitized at varying host densities were fitted to the appropriate functional response model using the nonlinear least squares regression procedure (PROC NLIN, SAS Institute 1999) to estimate the parameters *a* and T_h.

Parasitism rate, or the proportion of parasitized hosts, was determined by dividing the number of mummies by the initial host density. Sex ratio was calculated as the proportion of males among the offspring. Emergence rates were estimated by dividing the number of progeny emerged over the sum of the number of emerged progeny and the number of progeny failed to emerge. Proportion of males and emergence rate were arcsine-transformed to equalize data variance before statistical analyses. Analysis of variance (ANOVA, PROC GLM; SAS Institute 1999) was used to analyze the effects of host density on number of mealybugs parasitized, parasitism rate, emergence rate, progeny numbers, sex ratio and brood sizes of *A. loecki*. When significant differences were detected by ANOVA, a Tukey's honestly significant difference (HSD) test was used to separate the means (SAS Institute 1999).

Results

As the number of Madeira mealybugs increased from 2 to 50, the average number of mummies recovered from each replicate increased from 1.7 to 16.6 ($F = 86.23$, df = 6, 268, $P < 0.0001$) (Fig. 4.1A). The parasitism rate, however, decreased from 86% to 27% within the same range of host densities ($F = 60.28$, df = 6, 272, $P < 0.0001$) (Fig. 4.1B). The decrease in proportion of parasitized hosts was greatest between the densities of 2 to 20 mealybug per arena. The parasitism rates were not significantly different among the host densities greater than 20 mealybugs per arena.

The functional response of *A. loecki* foraging in arenas containing 2 to 50 mealybugs fitted a type II functional response (Table 4.1). Analysis of maximum likelihood reported a negative linear parameter (N_o) and a negative quadratic parameter (N_o^2), suggesting that the proportion of parasitized mealybugs decreased as the mealybug density increased. By fitting a type II functional response model to the data, the instantaneous attack rate (a) was estimated at 0.0303±0.0034 and the handling time was 1.0045±0.1121 h. Plots of the logistic regression equation and functional response model using estimated parameters are presented in Fig. 1, showing a high degree of fit between the observed and predicted data.

Host density significantly influenced the emergence, progeny production and sex allocation by *A. loecki* (Table 4.2). Progeny emergence rate was the lowest at a host density of 2 mealybugs per arena (mean = 89%). No significant difference was observed in emergence rate between the host densities of 5 to 50 mealybugs per arena, with more than 96% of all progeny emerging. The total number of progeny produced by a single female increased from 6 parasitoids at 2 mealybugs per arena to 31 parasitoids at 50 mealybugs per arena, representing a

500% increase. The proportion of males ranged between 0.3 to 0.4 among the varying mealybug densities, with the highest proportion of males in the lowest host density treatment.

Female *A. loecki* produced significantly larger broods in low host density treatments (Table 4.3). The average brood size was 3 progeny per mummy at the host density of 2 mealybugs per arena, and decreased to 2.5 at 20 mealybugs per arena and 1.8 at 50 mealybugs per arena. The numbers of female and male progeny per mummy reflected the pattern observed in the total brood size: the number of females decreased from 1.8 to 1.3 and the number of males decreased from 1.2 to 0.5 as the host density increased from 2 to 50 mealybugs per arena.

Discussion

Anagyrus loecki exhibited a type II functional response when foraging in patches with varying numbers of Madeira mealybug. Type II functional response is most frequently demonstrated in parasitoids, whereas type III occurred occasionally and type I in only a few parasitoid genera (Mills and Lacan 2004). The type I functional response observed in *Trichogramma* (Hymenoptera: Trichogrammatidae) and *Eretmocerus* (Hymenoptera: Aphelinidae) was suggested as a result of shared phylogenetic tendency to avoid superparasitism and egg depletion (Mills and Lacan 2004). Predators and parasitoids that have learned to search for prey or hosts more effectively, either by formation of search images or through use of kairomones, are more likely to exhibit the type III functional response (Fujii et al. 1986).

Hassell et al. (1977) argued that the type III functional response might be more common than had been demonstrated. The relative rarity of type III functional response may be an experimental artefact (van Lenteren and Bakker 1978). Under more natural conditions or in laboratory experiments with unrestricted movement, parasitoids would leave the experimental arenas when hosts available for parasitism were depleted. On the other hand, in laboratory

experiments where the parasitoids are confined in a patch for the entire experimental duration and forced to revisit parasitized hosts, type II functional response was more likely the result. Collins et al. (1981) and Sagarra et al. (2000) provided empirical data supporting this argument. When the mealybug parasitoid *Anagyrus kamali* Moursi was allowed to determine its residence time in an experimental arena, the parasitoid exhibited a type III functional response (Sagarra et al. 2000). In contrast, parasitoids enclosed within arenas for the entire experimental duration showed a type II functional response. Collins et al. (1981) obtained similar results using the aphid parasitoid *Aphelinus thomsoni* Graham (Hymenoptera: Aphelinidae). This study may also have suffered from the same weakness of functional response studies conducted in laboratory conditions. The reported type II functional response exhibited by *A. loecki* in this study may be the result of restricted movement in an enclosed arena. In addition, the higher brood size in the lower host densities (Table 4.3) suggested that the parasitoids were forced to revisit and superparasitize the same hosts.

The reproductive patterns of *A. loecki* changed with increasing host density. Due to the increased availability of hosts, female *A. loecki* produced significantly more progeny, both male and female, at the higher host densities. A plateau in the number of progeny was observed between the host densities of 20 to 40 mealybugs per arena. Numerical response studies on encyrtid parasitoids of mealybugs are rare. Sagarra et al (2000) studied the numerical response of *A. kamali* when the parasitoids were foraging in six host densities between 2 and 100 individuals of the mealybug *Maconellicoccus hirsutus* Green. *Anagyrus kamali* produced increasing progeny as the host density increased from 2 to 20 mealybugs per leaf, the number of progeny reached a plateau at higher host densities.

The sex ratio of *A. loecki* averaged 0.29 to 0.31 within the host densities of 10 to 50 mealybugs per arena. The proportion of males was significantly higher at the lowest host density. The sex ratio of *A. kamali* was not significantly different among the six host densities and the proportion of males averaged 0.3 (Sagarra et al. 2000). The difference in the sex allocation patterns between solitary and gregarious parasitoids at varying host density was most clearly demonstrated by Lysyk (2004). The gregarious *Trichomalopsis sarcophagae* Gahan (Hymenoptera: Pteromalidae) produced increasingly more female progeny at higher densities of its hosts, house fly (*Musca domestica* L.; Diptera: Muscidae) pupae. In contrast, the sex ratio of the solitary parasitoids *Muscidifurax raptor* Girault & Sanders and *Muscidifurax zaraptor* Kogan & Legner (both Hymenoptera: Pteromalidae) remained unchanged or only slightly increased within the same host density and on the same host. These results suggest that gregarious parasitoids may have the tendency to produce more male progeny at lower host density. Sibling mating among gregarious parasitoids is common. A reproductive female parasitoid that produces fewer male progeny faces increased risk of mating failure by her female progeny. Higher male progeny production at low host density ensures the successful mating and fertilization of her female progeny by their male siblings. The constraints in producing fewer male progeny, which are reproductively less profitable than female progeny, may break down in *A. loecki* when the host density decreases to a threshold of 10 mealybugs per patch, as suggested by the gradual decrease in sex ratio between 2 and 10 mealybugs per parasitoid.

The increased total progeny production was accompanied by a decrease in brood size. The smallest brood sizes were produced in the higher host densities, suggesting that the female parasitoids were able to adjust the clutch size (i.e., the number of eggs deposited per host) according to host availability. Brood sizes of the gregarious fly parasitoids *T. sarcophagae* and

Muscidifurax raptorellus Kogan & Legner (both Hymenoptera: Pteromalidae), which were obtained from pupae of the house fly, decreased with increased host density (Lysyk 2004). The largest brood sizes for the fly parasitoids were found when host density was less than 10. The brood sizes reach a stable level of parasitoid per mummy when host density exceeded 10 fly pupae per arena.

The emergence rates of *A. loecki* were above 95% in all host densities except the lowest density. The lower emergence rate at 2 mealybugs per arena may be related to the increased competition among siblings in the larger broods at this host density. The number of *A. kamali* that emerged at varying host densities followed a sigmoid pattern with the lowest numbers from the lower densities and overall averaged 26% (Sagarra et al. 2000). The emergence rates of the 4 species of fly parasitoids studied by Lysyk (2004) similarly increased from low to high host density until a plateau was reached.

The ability of a parasitoid to control a pest population is dependent on the parasitoid's functional and numerical response. O'Neil (1997) questioned the used of laboratory-derived functional response in assessing the effects of natural enemies on pest populations in the field. Despite the shortcoming of producing unnatural experimental conditions and the inaccuracy when compared to field measurements, functional and numerical response studies conducted in laboratories still provide valuable information or insights into the host-parasitoid interactions and thus are important to biological control (Houck and Strauss 1985). Results of this study suggested that *A. loecki* caused inverse density-dependent mortality in the Madeira mealybug population. *A. loecki-P. madeirensis* population dynamics may not be stable and thus the control of Madeira mealybugs by *A. loecki* may not be sustainable.

Host density is only one of the myriad of factors influencing the behavior and reproduction of natural enemies. Other biological and environmental factors, such as temperature (Jones et al. 2003), habitat spatial and structural heterogeneity (Ives et al. 1999), individual variations in foraging efficiency (Ives et al. 1999), natural enemy density and mutual interference (Jones et al. 1999, Mills and Lacan 2004), migration (Zemek and Nachman 1998), prey or host size preference (Aljetlawi et al. 2004), and alternative hosts (Lester and Harmsen 2002), could have significant impacts on the attack rate and thus the functional, numerical and developmental responses of the natural enemies. As shown by Lester and Harmsen (2002) a predator's functional and numerical responses do not always indicate the most effective biological control agent. The reproductive behaviors of predators and parasitoids are subjected to many factors other than host density. A comprehensive evaluation of the effectiveness of a natural enemy should involve testing the responses of the natural enemy to some of the above biotic and abiotic factors. Such comprehensive studies may also help to better predict the functional and numerical responses of natural enemies in the field situations.

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Table 4.1. Results of the analysis of maximum likelihood estimates by PROC CATMOD.

Parameter	Estimate ± SE	χ^2	df	*P*-value
Intercept (P_0)	1.3548 ± 0.2000	45.90	1	< 0.0001
P_1	-0.0722 ± 0.0272	7.07	1	0.0078
P_2	-0.00059 ± 0.00105	0.32	1	0.5732
P_3	0.000025 ± 0.000012	4.18	1	0.0410
Likelihood Ratio		648.84	275	< 0.0001

Table 4.2. Means (± SEM) of the number, proportion of males and emergence rates of progeny produced by each female *A. loecki* at varying mealybug density.

Mealybug density	Number of progeny			Sex ratio	Emergence
	Female	Male	Total	(Prop. male)	rate
2	3.5 ± 0.4d	2.2 ± 0.3c	5.7 ± 0.5d	0.40 ± 0.04a	0.89 ± 0.04b
5	6.6 ± 0.5d	3.5 ± 0.3bc	10.0 ± 0.6d	0.33 ± 0.02ab	0.97 ± 0.02a
10	11.5 ± 0.7c	4.6 ± 0.4b	16.2 ± 0.9c	0.29 ± 0.02b	0.97 ± 0.01a
20	15.4 ± 1.1bc	6.9 ± 0.7a	22.3 ± 1.4b	0.31 ± 0.02b	100a
30	17.6 ± 1.2ab	6.8 ± 0.5a	24.5 ± 1.5b	0.30 ± 0.03b	0.98 ± 0.01a
40	16.3 ± 1.4b	6.3 ± 0.7ab	22.6 ± 1.8b	0.30 ± 0.03b	0.98 ± 0.01a
50	22.1 ± 1.3a	8.6 ± 0.6a	30.7 ± 1.6a	0.29 ± 0.02b	0.97 ± 0.01a
ANOVA *F*	38.35	17.70	43.11	2.83	2.40
P > *F*	< 0.0001	< 0.0001	< 0.0001	0.0109	0.0282

Means followed the same letter within a column were not significantly different (Tukey's HSD, $\alpha = 0.05$, SAS Institute 1999).

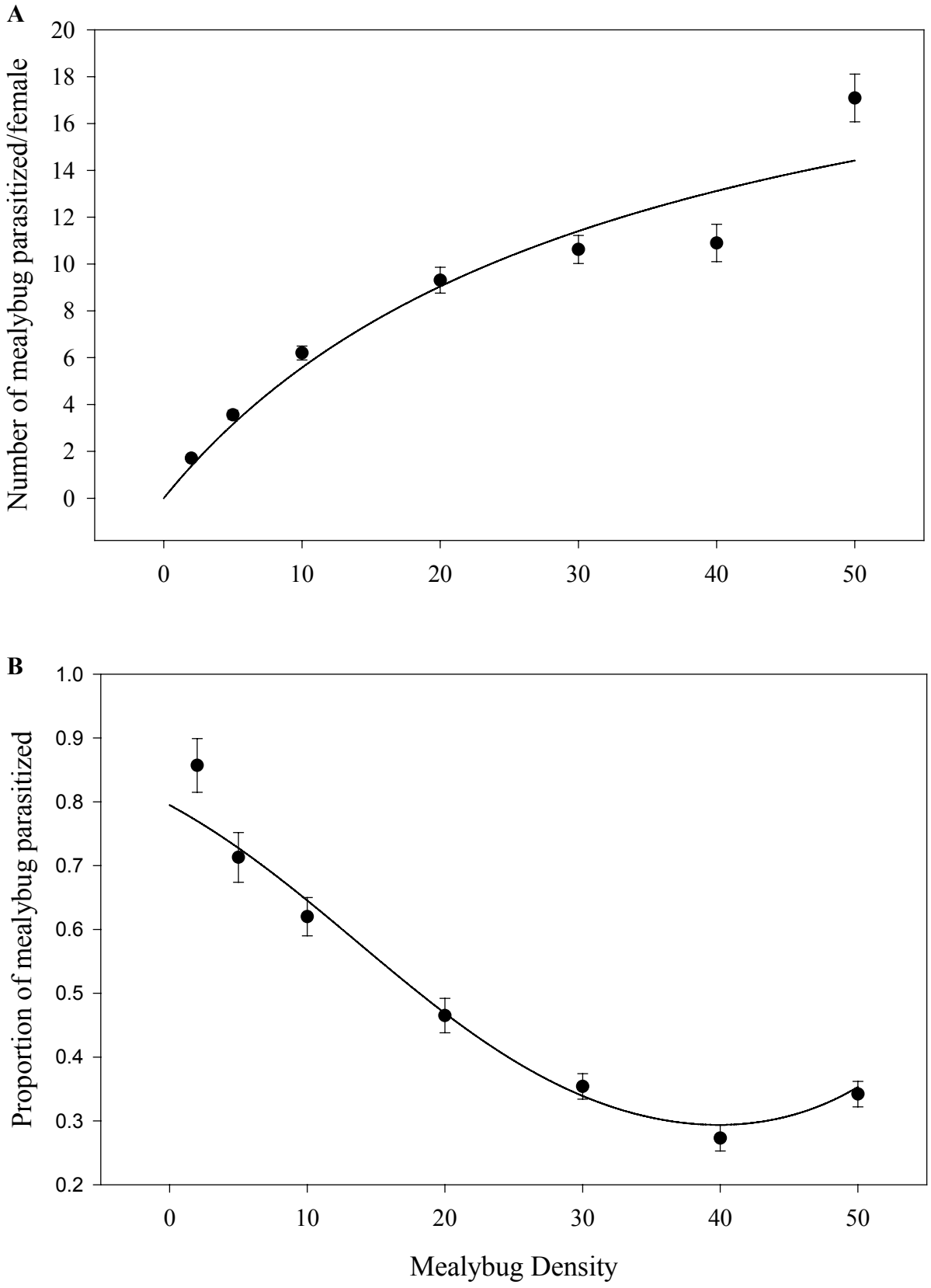
Table 4.3. Means (± SEM) of brood sizes of *A. loecki* emerged from each mummy of *P. madeirensis* at varying mealybug density.

Mealybug density	Number of progeny emerged per mummy		
	Female	Male	Total
2	1.8 ± 0.2a	1.2 ± 0.2a	3.1 ± 0.2a
5	1.8 ± 0.1a	0.9 ± 0.1ab	2.7 ± 0.1ab
10	1.9 ± 0.1a	0.8 ± 0.1bc	2.7 ± 0.1ab
20	1.7 ± 0.1ab	0.8 ± 0.1bc	2.5 ± 0.1bc
30	1.6 ± 0.1ab	0.7 ± 0.1c	2.3 ± 0.1cd
40	1.5 ± 0.1b	0.7 ± 0.1c	2.1 ± 0.1d
50	1.3 ± 0.1c	0.5 ± 0.1d	1.8 ± 0.1e
ANOVA *F*	15.09	18.20	26.85
P > *F*	< 0.0001	< 0.0001	< 0.0001

Means followed by the same letter within a column were not significantly different (Tukey's HSD, $\alpha = 0.05$, SAS Institute 1999).

Figure legends

Fig. 4.1. Number (A) and proportion (B) of mealybug parasitized by *A. loecki* at varying mealybug densities. The solid dots represented the means and the bars represented the SE of the measurements. The solid lines showed the values predicted by the functional response (in A) and logistic equations (in B).


A
Number of mealybug parasitized/female
0
2
4
6
8
10
12
14
16
18
20
0
10
20
30
40
50
B
Proportion of mealybug parasitized
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
10
20
30
40
50
Mealybug Density

CHAPTER 5

CONCLUSIONS

Van Lenteren and Woets (1988) proposed six desirable attributes of biological control agents for augmentative releases in greenhouses. The candidate natural enemy should be able to complete development and reproduce on the target pests in the climatic conditions of the intended release sites. The selected natural enemy should also exhibit high searching efficiency and possess a population growth rate higher than that of the target pests. The candidate biological control agent should be easy to rear and with no known non-target effects.

The goal of this doctoral dissertation is to evaluate the potential of the parasitoid, *Anagyrus loecki* Noyes & Menezes (Hymenoptera: Encyrtidae), as a biological control agent of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). Three aspects of the biological and ecological interactions between *A. loecki* and *P. madeirensis* were studied:

1) the effects of temperature, mating status and food supplements on the development, survival and reproduction of *A. loecki* on *P. madeirensis*;
2) the host stage preference and suitability of *P. madeirensis* for *A. loecki*; and
3) the functional and numerical responses of *A. loecki* in varying densities of *P. madeirensis*.

I will discuss the results of these studies in light of the selection criteria by van Lenteren and Woets (1988) and show that *A. loecki* is potentially an effective biological control agent of *P. madeirensis*.

Phenacoccus madeirensis has enormous growth and reproductive potentials. This mealybug species is able to complete development between 15 and 30^{o}C (Chong et al. 2003, 2004). Each female mealybug produced up to 600 eggs in a week. I estimated the lower developmental threshold and thermal constant to be 7.3^{o}C and 540.5 Degree-Days (DDs),

respectively. The high reproductive potential and immature survivorship allow a *P. madeirensis* population to reach a damaging level within a short period of time.

Anagyrus loecki is an arrhenotokous parasitoid, with the virgin female parasitoids producing only male progeny. *Anagyrus loecki* has an average lifetime fecundity of 78 progeny. The survival rate of the parasitoid larvae was above 94% between 15 and 30^{o}C. This parasitoid was able to complete development in *P. madeirensis* between 15 and 30^{o}C, which is the normal temperature range within the greenhouse. The developmental rate of the mealybug parasitoid increased with each incremental increase in temperature. The lower development threshold of *A. loecki* was estimated at 11^{o}C using a linear thermal summation model. The upper developmental threshold appeared to be between 30 and 35^{o}C because no parasitoid larvae survived to adulthood at 35^{o}C. The complete development of female parasitoids required 227.3 DDs where as the males required 217.4 DDs. These results suggest that *Anagyrus loecki* is able to complete development and reproduce in *P. madeirensis* within a temperature range favorable to the *P. madeirensis* population, satisfying two requirements suggested by van Lenteren and Woets (1988).

Although the lifetime fecundity of *A. loecki* is lower than that of *P. madeirensis*, this disadvantage is compensated for by the shorter developmental time of the parasitoid. At 25^{o}C, *A. loecki* was able to complete development in 16.5 days whereas the Madeira mealybug completed development in 29.8 days (Chong et al. 2003). In addition, *A. loecki* was able to parasitize and develop in all developmental stages of the mealybug hosts. Based on the biological information, I believe that one generation of *P. madeirensis* is able to support the development of two generations of *A. loecki*. The impact of *A. loecki* on a Madeira mealybug population should be compounded within a host generation. The definitive evidence on the

impact of *A. loecki* on *P. madeirensis* populations could come from studies on the population growth rates of both the parasitoid and mealybug or the kill rate of the mealybug by the parasitoid. Unfortunately, such study was not conducted in this doctoral dissertation research. Another factor that influences the host-parasitoid population dynamics is host feeding (Godfray 1994). During this dissertation research, I did not observe host feeding behavior by *A. loecki* in any of the experiments conducted. However, this behavior was observed in the greenhouses and during the course of another laboratory experiment not included in this dissertation. Future comprehensive evaluation of the potential and effectiveness of *A. loecki* against *P. madeirensis* should include a comparative study on the effects of temperature and host feeding on the population growth rate of the parasitoid and that of the mealybug in the presence of the parasitoid.

Anagyrus loecki is effective in searching for and parasitizing *P. madeirensis*. Foraging behavioral observations on *A. loecki* suggested that the parasitoid was able to parasitize and develop in all developmental stages of *P. madeirensis* but preferred the third-instar immature and pre-reproductive adult female mealybugs. 55 and 75% of the third-instar nymphs and pre-reproductive adult female mealybugs in a mixed culture of different developmental stages were examined by *A. loecki*, respectively. This result suggested *A. loecki* was especially effective in searching for its most preferred host stages. *Anagyrus loecki* was also a persistent pursuer. On several occasions during the behavioral study, the parasitoids pursued and eventually parasitized the escaped mealybugs. Defensive behaviors exhibited by the mealybugs were ineffective in deterring the parasitoids from examining and parasitizing them.

An encountered and examined *P. madeirensis* was almost always parasitized by *A. loecki*. The mealybugs never encapsulate the deposited parasitoid eggs. Thus, the parasitism

rate, as calculated by the proportion of hosts mummified, was a reliable indicator of the searching and parasitism efficiency. The life history study suggested that searching and parasitism efficiency was influenced by the temperature and mating status of the parasitoids. Rising temperature may have increased the foraging activities and egg maturation rate of *A. loecki* and contributed to the increased parasitism rates of *P. madeirensis* from 17% at 15^{o}C to 33% at 25^{o}C. Virgin parasitoids were more active in parasitizing *P. madeirensis* than the mated parasitoids. I did not investigate the behavioral and physiological basis for the difference in parasitism efficiency between virgin and mated parasitoids. As a result, I cannot offer an explanation for the observed difference between parasitoids of the two mating statuses.

Host density also influenced the parasitism rate of *A. loecki*. *Anagyrus loecki* exhibited type II functional response, meaning that the parasitism rate decreased exponentially with increase in *P. madeirensis* density. Functional response analyses are often used in comparing two or more candidate biological control agents. The most effective biological control agent is expected to be one that exhibits type III functional response (Murdoch and Oaten 1975). Type II functional response indicates an inversely density-dependent relationship between the parasitoid and the host populations, and thus is not able to produce a stable population dynamics. Based on this theoretical prediction, *A. loecki* is not expected to be an effective biological control agent of *P. madeirensis*. A *P. madeirensis* biological control program using only *A. loecki* will not be sustainable due to the frequent outbreaks and crashes in the host and parasitoid populations predicted by an inversely density-dependent population dynamics. However, the results of the functional response study on *A. loecki* should be interpreted with care. This *A. loecki* functional response study was conducted in closed Petri dishes. Similar to many laboratory studies, this study on the functional response of *A. loecki* may have suffered from the artificiality of

laboratory experiment (van Lenteren and Bakker 1978). Parasitoids studied under more natural laboratory conditions or in opened Petri dishes often exhibit type III functional response, and those with parasitoid movement restricted in enclosed arenas often exhibit type II functional response. Sagarra et al. (2000) reported the effect of experimental design on the functional response of the mealybug parasitoid *Anagyrus kamali* Moursi and provided empirical evidence for the above argument. To better predict the impact of *A. loecki* on *P. madeirensis* populations, the functional response of the parasitoid should be studied under more natural laboratory conditions or in the greenhouses where the parasitoids are allowed to determine their movement and residence time.

Biological control in greenhouse ornamental productions is characterized by the diversity of plants and pests. A biological control program for one pest must be compatible with the production practices and the management program against another pest. The non-target effects of a biological control agent on other beneficial or non-pest organisms have to be investigated. Study on the host range of *A. loecki* has been conducted but it is not included in this research project. Results of the host specificity study suggest that *A. loecki* is specific to *P. madeirensis* among the six mealybug species tested [*Ferrisia virgata* (Cockerell), *P. madeirensis*, *Phenacoccus solani* Ferris, *Planococcus citri* (Risso), *Pseudococcus longispinus* (Targioni Tozzetti) and *Pseudococcus viburni* (Signoret)] (Chong and Oetting, manuscript in preparation). The effect of *A. loecki* on non-target mealybug species is therefore expected to be minimal. The interactions between *A. loecki* and other biological control agents of mealybugs should also be studied.

Cultures of *A. loecki* can be easily established in the greenhouses, laboratories and insectaries. To establish viable colonies and produce sufficient number of the parasitoids, I

developed laboratory and greenhouse rearing methods. Prior to the establishment of an *A. loecki* colony in the greenhouses, host plants (coleus, eggplants or chrysanthemums) were infested with pre-ovipositing adult females and ovisacs of *P. madeirensis* collected from either another greenhouse colony or an insectary colony. The mealybugs were allowed to develop under greenhouse conditions for 3-4 weeks before introducing the parasitoids. Sprouted russet potatoes were used as host plants for the laboratory colonies. The potatoes were sprouted in closed styrofoam boxes and infested with ovisacs collected from an established mealybug colony maintained in the insectary. The mealybug colonies were kept in the insectary and only moved to the laboratory when needed. Laboratory colonies were established by releasing adult parasitoids into a cage containing the mealybug colonies maintained on sprouted potatoes. One week after the release of *A. loecki* into the greenhouse or laboratory colonies, the mealybug populations were examined for parasitoid mummies. These mummies were isolated in individual gelatin capsules and incubated until adult parasitoid emergence. Adult parasitoids were collected from the greenhouse or laboratory colonies 2-3 weeks after the introduction of the founder population.

The goal of a mass rearing program is to produce large numbers of high-quality biological control agents in an insectary for augmentative releases. Study of the host stage suitability for *A. loecki* suggested that parasitism in the third-instar immature and pre-reproductive adult female mealybugs produce the higher number of progeny with a female-biased sex ratio. Thus, commercial production of *A. loecki* should use third-instar or adult female *P. madeirensis* as hosts. It is tempting to shorten the production cycle by using young nymphs. However, such rearing practices will reduce the efficiency of the rearing program and

produce undesirable products. The young mealybugs produce smaller progeny, higher proportion of males and longer developmental time.

Carbohydrate- or protein-rich food sources are often provided to the adult or larval biological control agents during shipment. The biological control agents are often shipped in cool storage. Provisions of carbohydrate sources could significantly increase the longevity of the biological control agents. The longevity and reproductive potential of *A. loecki* were significantly increased when they were provided with carbohydrates in the form of honey solution. The longevity of honey-fed parasitoids was 6 to 14 times longer than that of starving individuals. The longevity of adult parasitoids was also influenced by storage temperature, with the honey-fed parasitoids stored at 15^{o}C lived 46 days or 9 times longer than the parasitoids fed with the same food sources at 35^{o}C. At 25^{o}C, the honey-fed *A. loecki* produced 7 times more progeny than the starved parasitoids. The longevity of *A. loecki* can be extended by storing the parasitoids at 15^{o}C and providing them with 50% honey solution. The effect of food supplements and cool storage during shipment on the efficiency of *A. loecki* has yet to be studied.

Based on the results from my doctoral dissertation research, *A. loecki* is clearly a suitable biological control agent against *P. madeirensis*. *Anagyrus loecki* developed, survived and reproduced on *P. madeirensis* within a temperature range that is also favorable for the development of the target host. The parasitoid has a high searching efficiency and parasitism ability against all development stages. The parasitoid is easy to rear and the colonies are easy to maintain. The non-target effect of *A. loecki* is minimal.

I propose the following considerations when releasing *A. loecki* for the control of *P. madeirensis*. The release of *A. loecki* should be synchronized with the life cycle of the target

pest so that the mealybug population consists of mostly third-instar nymphs or adult females. *Anagyrus loecki* that develop in the most suitable host stages may achieve higher rates of parasitism, survival and development, and produce a higher number of progeny consist of mainly female parasitoids. The mean temperature of the greenhouse should be maintained at 15 to 30^{o}C for the parasitoids to achieve the highest developmental rate. Choosing the appropriate release time and environmental conditions can enhance the establishment and effectiveness of the parasitoid population. Although *A. loecki* may not establish a long-term control against the Madeira mealybugs, the parasitoids can be released as an inundative or seasonal inoculative biological control agent when the mealybug population level is low. When the mealybug population is high, chemical control may be required to reduce the mealybug population below damaging level before the parasitoids can be released. Insecticides of choice may include insect growth regulators and other compatible chemicals. The list of compatible insecticides has yet to be determined. Provisions of carbohydrate food sources may help to conserve and increase the efficiency of *A. loecki* when the amount of honeydew produced by the low numbers of mealybug is insufficient.

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