

4266

UNIVERSITY OF HAWAI'I LIBRARY  
MASS PRODUCTION OF *ANAGYRUS ANANATIS* GAHAN (HYMENOPTERA:  
ENCYRTIDAE) FOR THE AUGMENTATIVE BIOLOGICAL CONTROL OF PINK  
PINEAPPLE MEALYBUG *DYSMICOCCLUS BREVIPES* (COCKERELL)  
(HOMOPTERA: PSEUDOCOCCIDAE)

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE  
UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

IN  
ENTOMOLOGY

DECEMBER 2002

BY  
RAJU RAJ PANDEY

DISSERTATION COMMITTEE:

Marshall W. Johnson, Chairperson  
Russell H. Messing  
Neil J. Reimer  
John S. Hu  
Andrew D. Taylor

© Copyright 2002

by

Raju R. Pandey

iii

## Dedication

Dedicated to the farmers of Nepal who encouraged me to conduct pest management studies in their farms during my early stage of entomology career.

## ACKNOWLEDGEMENTS

I like to express special thanks to my major Advisor Marshall Johnson for his continuous guidance, encouragement, support and patience throughout this work. Thanks to my committee members Drs Russell Messing, John Hu, Neil Reimer and Andy Taylor for their interest, suggestions and support to conduct the research work. Research was supported by USDA- CSRESS grant.

It would have been impossible for me to pursue graduate study at the University of Hawaii without the Scholarship from East West Center. Dr Ron Mau provided opportunity to work as a teaching assistant. Dr Cerruti Hooks was a great help in many ways. Mike Nomura helped maintain mealybug and the parasitoid culture so that I could spend more time in conducting research. He also helped in field works.

Drs Brent Sipes and Koon Hui Wang allowed to conduct some of my field research in their field. Gerath Nagai helped to maintain the field study sites. All three pineapple companies (Dole, Del Monte and Maui pine and Land companies) provided me unrestricted access to their fields to collect insects required for my study. Drs Gary Carr and Cliff Morden (Botany) helped to identify weeds. I am grateful to all the faculty, staff and graduate students of the department for their help and friendship. Special thanks to Mildred Uegawachi and Lynn Hata for keeping track of all the paper works.

Moral support of my family members back in Nepal and the Nepalese community in Honolulu was important. Love, patience and understanding of my wife Sancheeta, son Suveg and daughter Subigya made it possible to undertake this journey.

## ABSTRACT

Pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae), transmits Pineapple Mealybug Wilt Associated Virus (PMWaV) (Closteroviridae: Closterovirus) in Hawaii. In the presence of PMWaV, feeding by PPM induces Mealybug Wilt of Pineapple, which can completely devastate a pineapple planting if PPM is not managed. Studies were conducted to develop an augmentative biological control program for PPM management using the encyrtid endoparasitoid *Anagyrus ananatis* Gahan.

Use of vermiculite in PPM rearing protocols effectively removed PPM generated honeydew that commonly entrapped mealybugs and interfered with collection of PPM individuals from squash. Infestation of Kobocho squash with mature adult PPM produced more than 2200 mature adult PPM ( $\geq 0.6$  mg) per kg squash in about 8 weeks. The size of parasitized PPM significantly affected *A. ananatis* body size, which partially determined the parasitoid's reproductive ability. PPM weighing  $\geq 0.6$  mg produced normal sized *A. ananatis*.

The lower developmental threshold ( $T_0$ ) for *A. ananatis* was 12.65°C. Total heat accumulation required for development from egg to adult was 265 and 275 Degree Days (DD) for males and females, respectively. Eggs began hatching after accumulating 22 DD and larval development was completed after 88-110 DD. *Anagyrus ananatis* pupae can be stored at 10.1°C (below the  $T_0$ ) for one week, if preconditioned at 14.8°C (above the  $T_0$ ) for one week, without significant effects on their survival and reproductive abilities.

Big headed ant (BHA), *Pheidole megacephala* F. (Hymenoptera: Formicidae), effectively reduced *A. ananatis* parasitization of PPM to almost half of that recorded in its absence. Increased *A. ananatis* densities increased PPM parasitization in the presence of BHA, which suggested that augmentative field releases might help suppress PPM even when ants are present. The disruptive effects of BHA on *A. ananatis* parasitism could potentially be neutralized by doubling (via augmentative release) the densities of *A. ananatis* that provide effective PPM suppression in the absence of BHA.

The most common weed hosts of PPM included rhodes grass, *Chloris gayana*, and wire grass, *Eleusine indica*, which were found in both mowed and unmowed weed bands within disturbed areas around pineapple plantings. No mealybugs collected from these weeds produced parasitoids.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	v
ABSTRACT.....	vi
TABLE OF CONTENTS.....	viii
LIST OF TABLES.....	xii
LIST OF FIGURES.....	xv
I. INTRODUCTION.....	1
References.....	4
II. LITERATURE REVIEW.....	8
Arthropod Pests of Pineapple.....	9
Mealybugs as Crop Pests.....	10
Mealybug Wilt of Pineapple.....	12
<i>Dysmicoccus brevipes</i> (Cockerell).....	14
Pineapple Mealybug Wilt Associated Virus and Etiology of MWP.....	17
Ants and MWP.....	19
Pineapple Mealybug Wilt Control.....	21
Biological Control.....	24
Biological Control of Mealybugs.....	32
Factors Affecting Augmentative Biological Control.....	36
Biological Control Approaches for <i>Dysmicoccus brevipes</i> .....	37
Conclusions.....	38
References.....	40
III. ENHANCED PRODUCTION OF PINK PINEAPPLE MEALYBUG, <i>DYSMICOCCLUS BREVIPES</i> (COCKERELL), (HOMOPTERA: PSEUDOCOCCIDAE) .....	57
Introduction.....	57
Materials and Methods.....	61

Estimation of Honeydew Production .....	62
Suitability of Vermiculite and Two Squash Varieties for PPM Production .....	64
Suitability of Plastic and Paper Containers as Rearing Units .....	67
Optimum PPM Stage for Initial Squash Infestation.....	68
Results.....	70
Quantification of Honeydew Production.....	70
Suitability of Vermiculite and Two Squash Varieties for PPM Production .....	72
Suitability of Plastic and Paper Containers as Rearing Units .....	77
Optimum PPM Stage for Initial Squash Infestation.....	81
Discussion .....	86
References .....	92
IV. BIOLOGICAL PARAMETERS OF <i>ANAGYRUS ANANATIS</i> GAHAN (HYMENOPTERA: ENCYRTIDAE).....	96
Introduction.....	96
Materials and Methods.....	99
Factors Affecting Parasitoid Longevity .....	99
Periodicity of Biological Events .....	102
Results.....	104
Factors Affecting Parasitoid Longevity .....	104
Biological Events .....	111
Discussion .....	114
Conclusions.....	117
References .....	119
V. EFFECT OF PINK PINEAPPLE MEALYBUG HOSTS ON THE SIZE AND PROGENY PRODUCTION OF <i>ANAGYRUS ANANATIS</i> GAHAN (HYMENOPTERA: ENCYRTIDAE).....	125
Introduction.....	125
Materials and Methods.....	127
Influence of Mealybug Size on Parasitoid Size and Sex.....	127



Influence of Parasitoid Size on Progeny Production.....	129
Results .....	132
Influence of Host Size on Parasitoid Size and Sex .....	132
Influence of Parasitoid Size on Progeny Production.....	137
Discussion .....	143
Conclusions.....	148
References.....	149
 VI. DEVELOPMENT OF <i>ANAGYRUS ANANATIS</i> GAHAN (HYMENOPTERA: ENCYRTIDAE) AT CONSTANT TEMPERATURES.....	154
Introduction.....	154
Materials and Methods.....	157
Developmental Threshold .....	158
Biological Development of <i>Anagyrus ananatis</i> .....	161
Results.....	162
Developmental Threshold .....	162
Biological Development of <i>Anagyrus ananatis</i> .....	169
Discussion .....	173
Conclusions.....	179
References .....	181
 VII. STORAGE OF <i>ANAGYRUS ANANATIS</i> GAHAN (HYMENOPTERA: ENCYRTIDAE) IMMATURES AT COOL TEMPERATURES .....	186
Introduction.....	186
Materials and Methods.....	189
Survival of Cool Storage and adult longevity .....	189
Progeny Production .....	191
Results.....	192
Survival of Cool Storage .....	192
Identification of Stages Not Surviving Cool Storage.....	194
Effects on Developmental Time and Adult Longevity .....	196

Progeny Production .....	202
Discussion .....	202
Conclusions .....	207
References .....	209
VIII. THE IMPACT OF BIG HEADED ANT ON <i>ANAGYRUS ANANATIS</i> GAHAN (HYMENOPTERA: ENCYRTIDAE) PARASITISM OF THE PINK PINEAPPLE MEALYBUG.....	
Introduction.....	213
Materials and Methods.....	216
Results and Discussion.....	220
References.....	227
IX. SURVEY FOR PINK PINEAPPLE MEALYBUG AND ASSOCIATED PARASITOIDS IN WEEDS BORDERING PINEAPPLE PLANTINGS.....	
Introduction.....	232
Materials and Methods.....	235
Results .....	241
Discussion .....	253
References.....	255
X. SUMMARY AND RECOMMENDATIONS.....	
PPM Mass Production.....	258
Biological Parameters of <i>Anagyrus ananatis</i> .....	259
Overcoming the Impact of Ants .....	261
Survey of Weeds Around Pineapple Plantings .....	262
Future Research Recommendations .....	262

## LIST OF TABLES

Table 2. 1. Parasitoids introduced to Hawaii to control <i>Dysmicoccus</i> mealybugs.....	25
Table 2. 2. Predators introduced to Hawaii for the control of <i>Dysmicoccus</i> mealybugs ..	26
Table 3. 1. Production of pink pineapple mealybug on butternut and Kobocho squash varieties with and without vermiculite provided in the rearing container to absorb honeydew. ....	76
Table 3. 2. Effect of plastic and paper containers on the laboratory rearing of pink pineapple mealybug. ....	80
Table 3. 3. Effect of initial PPM infestation on PPM production in the laboratory. ....	87
Table 4. 1. Effects of water and honey on <i>Anagyrus ananatis</i> adult longevity. ....	105
Table 4. 2. Impact of honey concentration on adult female <i>Anagyrus ananatis</i> longevity. ....	106
Table 4. 3. Effect of starvation on adult female <i>Anagyrus ananatis</i> longevity. ....	107
Table 4. 4. Effect of prior feeding on honey on the progeny production and post- feeding longevity of <i>Anagyrus ananatis</i> .....	109
Table 4. 5. Photophase and scotophase influence on rate of <i>Anagyrus ananatis</i> oviposition as indicated by adult emergence from parasitized PPM. ....	110
Table 4. 6. Adult emergence of <i>Anagyrus ananatis</i> during specific time intervals.....	112
Table 5. 1. Effect of mealybug size and source on adult <i>Anagyrus ananatis</i> length. ....	133
Table 5. 2. Effect of mealybug host size on sex allocation by <i>Anagyrus ananatis</i> . ....	134
Table 5. 3 Relationship of PPM mummy size to the sex and body length of emerged <i>Anagyrus ananatis</i> . ....	138

Table 6. 1. Number of adult <i>Anagyrus ananatis</i> that completed development from egg to adult at various temperatures. ....	163
Table 6. 2. The lower temperature threshold, ( $T_o$ ) in °C and accumulated temperature requirement (K) in degree days (DD) of <i>Anagyrus ananatis</i> .....	166
Table 7. 1. Percent emergence of <i>Anagyrus ananatis</i> adults following cool temperature storage under various conditions during immature stages.....	193
Table 7. 2. Mean percentages of dead <i>Anagyrus ananatis</i> individuals that were found in the prepupal, pupal, and adult stages within PPM mummies following cool temperature storage of immature stages. ....	195
Table 7. 3. Mean time periods (days) required by <i>Anagyrus ananatis</i> immatures, following cool storage under various conditions, to eclose following incubation at $25 \pm 1^\circ\text{C}$ . ....	198
Table 7. 4. Estimated Degree-Days accumulated by <i>Anagyrus ananatis</i> immatures while held in cool storage under various conditions.....	199
Table 7. 5. Mean longevity (days) of <i>Anagyrus ananatis</i> adult females following cool storage during immature stages. ....	201
Table 7. 6. Mean number of progeny produced per day by <i>Anagyrus ananatis</i> females that previously underwent cool storage under various temperature and storage conditions during their immature stages. ....	203
Table 8. 1. Effect of ant attendance and screen barrier on PPM recovery <sup>1</sup> .....	222
Table 8. 2. Effect of ant attendance and female <i>A. ananatis</i> density on percentage PPM parasitization . ....	224

Table 9. 1. Percentage composition of weed flora in managed areas <sup>1</sup> on the paved roadway side of pineapple plantings.....	242
Table 9. 2. Widths of the unmowed and mowed weed bands and weed species density per survey site (1 m <sup>2</sup> ). .....	244
Table 9. 3. Frequency of major weeds in disturbed plant communities adjacent to borders of pineapple plantings near paved roadways. ....	246
Table 9. 4. PPM densities on various weed species adjacent to pineapple plantings. ....	248
Table 9. 5. Mean plant height and number of tillers per plant at various growth stages, and their correlation with total PPM and total tillers at seed dispersing stage per plant. ....	249
Table 9. 6. Number of adult mealybugs collected and observed for parasitoid emergence from various weeds during the survey.....	251
Table 9. 7. Number of mummies developed from mealybugs (MB) collected from <i>C. gayana</i> with and without exposure to <i>A. ananatis</i> in the laboratory. ....	252

## LIST OF FIGURES

Fig. 3. 1. Squash setup for studying honeydew production by PPM. Twenty adult PPM were allowed to feed on ventral squash surface.....	63
Fig. 3. 2. Experimental setup for the mass production of PPM. ....	65
Fig. 3. 3. Mean weight of honeydew (Mean $\pm$ SEM) produced by individual PPM adults per day. ....	71
Fig. 3. 4. Mean weight ( $\pm$ SEM) of honeydew produced by PPM colony reared on a squash over 10 weeks.....	73
Fig. 3. 5. Establishment and increase in the surface area covered by PPM as affected by two squash varieties (Butternut and Kobocho) and treatment (presence and absence of vermiculite). ....	75
Fig. 3. 6. Effect of plastic and paper containers holding PPM infested squash relative to initial establishment and size of surface area covered by PPM.....	78
Fig. 3. 7. Effect of initial PPM inoculum stage. ....	82
Fig. 3. 8. Mean weight (mg) of PPM individuals as affected by initial inoculum source. ....	84
Fig. 3. 9. Proportionate distribution of different PPM stages produced by different infestation sources and separated by standard sieves. ....	85
Fig. 4. 1. Adult emergence of <i>Anagyrus ananatis</i> during specific time intervals.....	113
Fig. 5. 1. Mean mummy length, width and their ratios of the mummies producing male and female <i>A. ananatis</i> .....	136
Fig. 5. 2. Effect of Mealybug mummy size on sex allocation by <i>Anagyrus ananatis</i> . ....	139

Fig. 5. 3. Relationship between female <i>A. ananatis</i> body length and progeny production. ....	140
Fig. 5. 4. Relationship between PPM host weight and <i>A. ananatis</i> body length produced therefrom. ....	142
Fig. 6. 1. Effect of rearing temperature on the time to complete development to adult stage of <i>A. ananatis</i> . ....	164
Fig. 6. 2. Effect of temperature on the developmental rate of <i>A. ananatis</i> .....	165
Fig. 6. 3. Change in the developmental rate of <i>A. ananatis</i> in relation to the increase in the rearing temperature. ....	167
Fig. 6. 4. Developmental stages of <i>A. ananatis</i> at constant temperature of $23.5 \pm$ $0.5^{\circ}\text{C}$ .....	170
Fig. 8. 1. Schematic diagram showing experiment setup. . . . .	219
Fig. 9. 1. Twenty-five pin point-quadrat.....	237

## I. INTRODUCTION

Several mealybug species (Homoptera: Pseudococcidae) infest pineapple, but three species are commonly encountered in the pineapple agroecosystem: pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell), gray pineapple mealybug (GPM), *D. neobrevipes* Beardsley, and long tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Beardsley 1959; German *et al.* 1992; Beardsley 1993). PPM and GPM are important pests because of their ability to vector Pineapple Mealybug Wilt Associated Virus (PMWaV) (Sether *et al.* 1998). The pineapple mealybugs in association with the PMWaV induce mealybug wilt disease of pineapple (MWP), the most destructive disease of pineapple worldwide (Illingworth 1931; German *et al.* 1992; Hughes and Samita 1998; Sether *et al.* 1998). PPM is one of the most widely distributed, tropicopoliotan mealybug species around the world, infesting more than 140 plant species (Williams and Willink 1992; Ben Dov 1994). Surveys conducted in several pineapple plantings on the Hawaiian Islands of Oahu and Maui suggested that PPM was the predominant mealybug infesting Hawaiian pineapples (Gonzalez-Hernandez *et al.* 1999b). Several weeds (e.g., grass and broadleaf species) commonly found around pineapple fields have been considered as a source for PPM in Hawaii (Carter 1933; Carter 1951; Bartlett 1978; Rohrbach and Apt 1986; German *et al.* 1992).

Various ant species (Hymenoptera: Formicidae) have developed intimate relationships with many Homopteran insects (e.g., aphids, mealybugs, scales, psyllids and plant hoppers) because the ants feed on the honeydew produced by these insects (Flanders 1951; Nixon 1951; Campbell 1994). The big headed ant, *Pheidole*



*megacephala* (F.), and other ant species such as Argentine ant, *Linepithema humile* (Mayr), and fire ant, *Solenopsis geminata* (F.), attend both PPM and GPM in Hawaiian pineapple (Illingworth 1931; Carter 1932; Rohrbach *et al.* 1988; Jahn and Beardsley 1998; Gonzalez-Hernandez *et al.* 1999a; Jahn and Beardsley 2000). A primary benefit of this association to the mealybugs is protection provided from their natural enemies (Rohrbach *et al.* 1988; Reimer *et al.* 1993; Jahn and Beardsley 1998; Gonzalez-Hernandez *et al.* 1999b). Other benefits include transportation and dispersal of mealybugs by ants, and increased survival as a result of removal of honeydew and construction of shelters (Illingworth 1931; Nixon 1951; Duodu and Thompson; Petty and Tutsin 1993).

More than 20 species of natural enemies of PPM have been introduced to Hawaii, (Sakimura 1939; Funasaki *et al.* 1988; Gonzalez-Hernandez *et al.* 1999b) of which at least five species have successfully established and provided partial control of PPM even in the presence of ants (Gonzalez-Hernandez *et al.* 1999b). Effective insecticidal control of mealybugs in pineapple is achieved either by: a) foliar applications of diazinon, a restricted use organophosphate insecticide, or b) controlling the ants that tend PPM and GPM (Rohrbach *et al.* 1988; Reimer and Beardsley 1990; Botrel *et al.* 1993). After the banning of most of the organochlorine insecticides (e.g aldrin, chlordane, heptachlor and mirex,) used for ant control, the only insecticide being permitted for ant suppression in Hawaiian pineapple is hydramethylnon. It is applied in a bait formulation (Amdro®, American Cynamid Co) under an emergency needs (Section 18) registration (Rohrbach *et al.* 1988; HDOA 2001). Alternative pest management techniques are necessary if these

pesticides become unavailable in the future. Additionally, no effective, non-pesticidal based management techniques exist currently for organic pineapple production systems.

*Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae) was established in Hawaii after being introduced from Brazil between 1935 -1937 (Funasaki *et al.* 1988; Sakimura 1939). This solitary endoparasitoid was found to be the most important biological control agent of PPM in pineapple with potential for use in an augmentative biological control program (Gonzalez-Hernandez *et al.* 1999b), but limited information is available on its biology (Gonzalez-Hernandez 1995). This parasitoid is not commercially available in the numbers required for augmentative field releases due to the lack of techniques to mass-produce it. PPM infestations are common in the weeds that surround pineapple plantings, but the existence and survival of this parasitoid and other biological control agents on PPM in alternative weed hosts have not been investigated.

The main objectives of this study were a) to develop a mass rearing technique for PPM to provide host material for *A. ananatis*; b) to develop a mass rearing methodology for the encyrtid *A. ananatis* for use in augmentative biological control; c) to better understand the biology of *A. ananatis* relative to the needs of an augmentative program (e.g., developmental thresholds, cold storage thresholds, importance of adult nutrition); d) to investigate *Dysmicoccus* mealybug and natural enemy distribution in weed hosts surrounding pineapple plantings; and e) to evaluate the impact of ants on the efficacy of *A. ananatis* as a parasitoid of PPM in the laboratory.

## REFERENCES

- Bartlett, B. R. 1978. Pseudococcidae, pp. 137-171. *In* Introduced Parasites and Predators of Arthropod Pest and Weeds: A World Review (C. P. Clausen Ed.), U. S. Department of Agriculture, Handbook No. 480, Washington D.C.
- Beardsley, J. W. 1959. On the taxonomy of pineapple mealybugs in Hawaii, with the description of previously unnamed species (Homoptera: Pseudococcidae). *Proceedings of the Hawaiian Entomological Society* 17: 29-37.
- Beardsley, J. W. 1993. The pineapple mealybug complex: taxonomy, distribution and host relationships. pp. 383-386. *In* "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First International Pineapple Symposium, 2-6 November, 1992 Honolulu, Hawaii.
- Ben Dov, Y. 1994. A Systematic Catalogue of the Mealybugs of the World (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with Data on Geographical Distribution, Host Plants, Biology and Economic Importance. Intercept, Andover, UK, 686 pp.
- Botrel, N., D. L. D. Siqueira and D. L. De Siqueira. 1993. Control of pineapple mealybug. *Pesquisa Agropecuaria Brasileira* 28: 223-227.
- Campbell, C. A. M. 1994. Homoptera associated with the ants *Crematogaster clariventris*, *Pheidole megacephala* and *Tetramorium aculeatum* (Hymenoptera: Formicidae) on cocoa in Ghana. *Bulletin of Entomological Research* 84: 313-318.
- Carter, W. 1932. Pineapple mealybug, *Pseudococcus brevipes* (Ckll), occurring on pineapple plants. *Ecology* 13: 296-304.

- Carter, W. 1933. The pineapple mealybug *Pseudococcus brevipes* and wilt of pineapple. *Phytopathology* 23: 207-242.
- Carter, W. 1951. The feeding sequence of *Pseudococcus brevipes* (Ckll) in relation to mealybug wilt of pineapple in Hawaii. *Phytopathology* 41: 769-780.
- Duodu, Y. A. and W. Thompson. 1992. Management of ant-mealybug complex in pineapple fields in Guyana. *FAO Plant Protection Bulletin* 40: 82-88.
- Flanders, S. E. 1951. The role of the ant in the biological control of homopterous insects. *Canadian Entomologist* 83: 93-98.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. *Advances in Disease Vector Research* 9: 241-259.
- Gonzalez-Hernandez, H. 1995. The Status of Biological Control of Pineapple Mealybugs in Hawaii. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Gonzalez-Hernandez, H., M. W. Johnson and N. J. Reimer. 1999a. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15: 145-152.
- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999b. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- HDOA. 2001. List of active SLN registration. Pesticide Branch, Hawaii Department of Agriculture, Honolulu, pp.
- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.

- Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Jahn, G. C. and J. W. Beardsley. 1998. Presence absence sampling of mealybugs, ants, and major predators in pineapple. *Journal of Plant Protection in the Tropics* 11: 73-79.
- Jahn, G. C. and J. W. Beardsley. 2000. Interactions of ants (Hymenoptera: Formicidae) and mealybugs (Homoptera: Pseudococcidae) on pineapple. *Proceedings of the Hawaiian Entomological Society* 34: 181-185.
- Nixon, G. E. J. 1951. *The Association of Ants with Aphids and Coccids*. CIE, London, 36 pp.
- Petty, G. J. and H. Tutsin. 1993. Ant (*Pheidole megacephala* F.) - Mealybug (*Dysmicoccus brevipes* Ckll.) relationship in pineapples in south Africa. pp. 387-395. In "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First international pineapple symposium, 2-6 Nov, 1992 Honolulu, Hawaii.
- Reimer, N. J. and J. W. Beardsley. 1990. Effectiveness of hydramethylnon and fenoxycarb for control of bigheaded ant (Hymenoptera: Formicidae), an ant associated with mealybug wilt of pineapple in Hawaii. *Journal of Economic Entomology* 83: 74-80.
- Reimer, N. J., M. Cope and G. Yasuda. 1993. Interference of *Pheidole megacephala* (Hymenoptera: Formicidae) with biological control of *Coccus viridis* (Homoptera: Pseudococcidae) in coffee. *Environmental Entomology* 22: 483-488.

- Rohrbach, K. G. and W. J. Apt. 1986. Nematode and disease problems of pineapple.  
Plant Disease 70: 81-87.
- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988.  
Mealybug wilt, mealybugs, and ants of pineapple. Plant Disease 72: 558-565.
- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by  
the experiment station of the pineapple producers cooperative association during  
the period 1930-1938. Proceedings of the Hawaiian Entomological Society 10:  
352.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug  
wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.).  
Phytopathology 88: 1224-1230.
- Williams, D. J. and M. C. G. D. Willink. 1992. Mealybugs of Central and South America.  
CAB International, Wallingford, 635 pp.

## II. LITERATURE REVIEW

'Of cultivated crops there are an endless number, and the first of these is the pineapple which, as the king of them all nature has crowned with a diadem of its own leaves. The later surround it with thorns and guard it like archers'  
--Sebastiao da Rocha Pitta -from (Young 1940)

Pineapple, *Ananas comosus* (L.) Merr. (Family Bromeliaceae, subclass Monocotyledons), originated in South America, probably in Brazil (Johnson 1935; Collins 1960). The name *Ananas* may have originated from the Guarani language where 'a' signified fruit and 'na-na' meant 'excelling' or 'fragrance-fragrance' (Johnson 1935; Young 1940). The Spanish called this fruit 'Pina' from its resemblance to a pinecone and the English added 'apple,' giving the name pineapple (Johnson 1935). Though the exact date is unknown, it is believed to have been introduced to Hawaii during the early 1800's, probably via Tahiti (Collins 1960).

Total pineapple production worldwide in 2000 was 13,444,000 metric tons, and Thailand, the Philippines, Brazil, China and India are among the major producers (FAO 2001). Hawaii once produced more than half of the world's pineapple (German *et al.* 1992), but its current share is less than 3 percent (FAO 2001). Pineapple remains the largest agricultural commodity in Hawaii, covering more than 8,000 ha of land and producing 323,000 tons of fruit each year, which is worth nearly 100 million dollars (Rohrbach *et al.* 1988; HASS 2002).

Pineapple is a perennial herb and grows in all tropical and sub-tropical regions worldwide (Johnson 1935; Collins 1960). Pineapples are commonly grown as high as 700 m above sea level in Hawaii (Collins 1960), but most of the commercial plantations are located below 600 m (Reimer and Beardsley 1990). Of the five major pineapple varieties (Spanish, Queen, Pernambuco, Perolera and Cayenne), Cayenne is the most

widely grown worldwide (Sinclair 1993). It is believed to have originated from Cayenne in French Guiana (Collins 1960). This variety has smooth, spineless leaves, high fruit quality, and high productivity (Sinclair 1993). Smooth cayenne is resistant to gummoses (German *et al.* 1992), but susceptible to mealybug wilt of pineapple (MWP) (Carter 1934; Carter and Collins 1947).

A plant crop, commonly grown from crowns, takes about 6-16 months to produce an inflorescence (German *et al.* 1992; Sinclair 1993). Application of chemicals such as ethylene, acetylene or auxins can artificially induce flowering, and has become a standard practice in the pineapple industry (known as ‘forcing’) to synchronize flowering and subsequent fruit harvesting (Py *et al.* 1987). Development of the fruit results in the loss of apical dominance, which leads to sucker development. The suckers can complete their fruit production cycle in about a year. Crops following the “plant crop” are referred to as ratoon crops (Sinclair 1993).

Mealybug wilt disease, root lesion nematodes (*Rotylenchus reniformis*), and butt rot (*Chalara* sp.) are among the most important production constraints of pineapple (Rohrbach and Apt 1986). Mealybug wilt disease is the result of a complex interaction among ants, mealybugs and closterovirus and is the most important pineapple problem worldwide (Carter 1963; Gunashinghe and German 1986; Hu *et al.* 1996; Hu *et al.* 1997).

### **Arthropod Pests of Pineapple**

Several insects (e.g., mealybugs, scales, lepidopterous larvae) and mites can cause considerable damage to pineapple crops, affecting both quality and quantity of production. Three mealybug species, pink pineapple mealybug, *Dysmicoccus brevipes* (Cockerell), gray pineapple mealybug, *D. neobrevipes* Beardsley, and long tailed



mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Homoptera: Pseudococcidae), are commonly encountered in pineapple (Illingworth 1931a; Beardsley 1959; Rohrbach *et al.* 1988). Ants do not cause direct crop loss in pineapples, but are a considerable nuisance because they tend the mealybugs and interfere with their biological control agents. Big headed ant, *Pheidole megacephala* (F.), Argentine ant, *Linepithema humile* (= *Irydomyrmex humilis*) (Mayr), and fire ant, *Solenopsis geminata* (F.), are the most common ants found in Hawaiian pineapples (Rohrbach *et al.* 1988; Reimer *et al.* 1990). Fruit and stem borers, *Thelca echion* (L.) and *Castnia licus* (Drury) (Lepidoptera: Castniidae), respectively, are important lepidoptera pests in other locations (Collins 1960). Other pests include symphyllids (*Scutigera* spp.), pineapple scale (e.g., *Diaspis* spp.) (Collins 1960; Py *et al.* 1987). The pineapple souring beetles, *Carpophilus hemipterus* L., *C. humeralis*, and *Heptoncus ocellaris* (Fairm) (Coleoptera: Netudillidae), are considered secondary pests, attracted to pineapples which are wounded or are infested with mealybugs (Collins 1960; Py *et al.* 1987). Long horned grasshopper, *Conocephalus saltator* (Saussure) (Orthoptera: Tettigonidae), was considered both as a predator of mealybugs as well as a pest of pineapple, especially when the fruit is developing (Illingworth 1931b). Mites such as *Dolichotetranychus floridanus* (Bank) and *Sterneotarsonemus ananas* (Tryon) have questionable roles in retarding plant growth, hastening leaf senescence, and transmitting and promoting some fungal diseases (Py *et al.* 1987).

### **Mealybugs as Crop Pests**

Mealybugs (Homoptera: Pseudococcidae) are soft-bodied, plant-feeding, terrestrial insects with piercing and sucking mouthparts and are among the most serious

plant pests (McKenzie 1967; Ben Dov 1994). About 2,000 mealybug species exist worldwide (Williams and Watson 1988). Like other homopteran insects, mealybugs cause crop damage by: 1) removing plant sap and impacting plant vigor (Herren 1994); (McKenzie 1967; Buckley 1987; Thippaiah and Kumar 1999); 2) secreting honeydew that supports sooty mold growth and reduces photosynthesis rates and cosmetic values (Nixon 1951; Buckley 1987; Goolsby *et al.* 2000); and 3) transmitting various plant pathogens (DeBach 1980; Buckley 1987; Lockhart *et al.* 1992). Some common pathogens transmitted by mealybugs include grapevine leafroll associated closterovirus, pineapple mealybug wilt associated closterovirus, rice chlorotic streak virus, and cacao swollen shoot virus (Garau *et al.* 1995; Adu-Ampomah *et al.* 1996; Abo and Sy 1998; Sether *et al.* 1998).

Mealybugs are seldom important economic pests where they are indigenous (Beardsley 1978). Because of their cryptic habit and small size, mealybugs disperse with commercial traffic, mostly on imported plant materials, and attain serious pest status when introduced to new locations (DeBach 1964b, 1980; Williams and Willink 1992). Most mealybug pests are successfully controlled by the introduction of natural enemies (DeBach 1964b; DeBach and Rosen 1991). Cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, was the target of one of the greatest and most successful biological control programs implemented in recent years. Its control had tremendous impact on the livelihood of millions of African farmers (Neuenschwander 2001). Similarly, control of the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green, in the Caribbean area is another successful example of mealybug biological control (Sagarra and Peterkin 1999).

On the other hand, only partial control has been achieved against pineapple mealybugs in Hawaii and elsewhere (Beardsley 1978; Gonzalez-Hernandez *et al.* 1999a).

*Mealybugs infesting pineapples.* Several mealybug species infest pineapples (Carter 1942; Williams and Willink 1992), but three are most commonly encountered in Hawaii: pink pineapple mealybug (PPM), gray pineapple mealybug (GPM), and long tailed mealybug (LTM) (Carter 1935a; Beardsley 1959, 1965; German *et al.* 1992). PPM and GPM are considered the primary vectors of MWP (Sether *et al.* 1998). Although GPM was more commonly associated with fruit infestation and wilt disease (Beardsley 1993), PPM was more prevalent in the pineapple crop (Gonzalez-Hernandez *et al.* 1999b). The presence of mealybugs on fruit also reduces the fruit's marketability and produces quarantine problems relative to exportation (Lee 1966; Beardsley 1993).

### **Mealybug Wilt of Pineapple**

Wilt diseases in pineapple were recognized long before their incidence in Hawaii, but they were attributed to poor soil conditions and fungi (Johnson 1935). Pineapple wilts were classified into at least four categories: a) starvation wilt chiefly from nitrogen deficiency; b) nematode wilt; c) swamp wilt, due to poor soil conditions; and d) quick wilt, a sudden collapse of large healthy plants generally after harvest of the plant crop, which was often called 'ratoon wilt' (Johnson 1935).

Larsen (1910) first recognized wilt of pineapple in Hawaii in 1910. He isolated three types of fungi from the roots of wilted plants. Because he could not prove the pathogenicity of these fungi, he ascribed the wilt to poor soil conditions leading to secondary fungal infections (Johnson 1935). Higgins (1912) reported that the wilt had not caused great damage except in a few fields. Soon after, the disease incidence greatly

increased and by the early 1920's wilt was regarded as the most important pineapple disease, almost wiping out Hawaii's pineapple industry (Illingworth 1931a).

Mealybugs were an important factor in the epidemiology of pineapple wilt because they transmitted the wilt from diseased plants to healthy ones (Illingworth 1931a). Because the wilt condition was associated with mealybug infestations, it was called the 'mealybug wilt disease' (Carter 1933a). Currently, MWP occurs in all pineapple production areas worldwide (Illingworth 1931a; Carter 1963; Hughes and Samita 1998). Because the disease first appeared on field edges or near rocky areas, progressing towards the center of a field, it was initially called "edge wilt" (Illingworth 1931a; Carter 1932a; Carter 1933a). Carter (1933a) showed a time-quantity relationship for mealybug feeding and development of wilt symptoms. He categorized the wilt into: 1) 'quick wilt' (symptoms appeared within two months due to the feeding of large numbers of mealybugs over a short period); and 2) 'slow wilt' (which developed after prolonged feeding of fewer mealybugs).

*MWP symptoms.* The first measurable symptom of MWP is the cessation of root elongation, followed by root death (Carter 1948). Subsequently, leaves begin to show wilting (Carter 1948). If a plant can regenerate its roots, it will reestablish leaf turgor and the plant will recover. However, affected plants have reduced weight, leaf surface, number, length and breadth as well as root length (Carter, 1962; Lim 1972).

Carter (1935b, 1973) described MWP development, which routinely appears in four visible stages, sometimes followed by a fifth stage termed the 'recovery stage.' Initial symptoms are the reddening and slight inward bowing of the leaf margin (Stage 1) followed by a change of color from red to pink (Stage 2). The leaf bows further,

followed by wilting of the leaf (Stage 3), leading to drying of the leaves from the tip downward (Stage 4). Later, infected plants wilt and may die if mealybugs are not controlled. Time from initial infection to appearance of symptoms depends upon the mealybug population size, crop growth stage, and plant parts infested.[Carter, 1962 #1407; Lee, 1966 #627; Lee, 1968 #629; Lim, 1972 #631]

PPM and GPM feeding causes “mealybug stripe.” Chlorotic patches develop at the feeding sites due to the depletion of leaf cells.[Carter, 1962 #1407] At PPM feeding sites, cell walls in the chlorotic area are thickened, being more pronounced in the center of the spot.[Carter, 1962 #1407] GPM feeding leads to the development of green spotting, due to the collapse of phloem cells and degeneration of chlorophyll. Green spotting is bizonate (i.e., deep green center surrounded by light green area, which is still darker than the normal leaf area).[Carter, 1962 #1407]

### ***Dysmicoccus brevipes* (Cockerell)**

The pink pineapple mealybug was first described from Jamaica in 1893 as *Dactylopius brevipes* Cockerell, and was later renamed *Pseudococcus brevipes* (Cockerell) Fernald (Williams and Willink 1992). Kotinsky and Ehrhorn reported this insect as *Pseudococcus bromeliae* Bouche in 1910 from Hawaii (Kotinsky 1910). Because *P. bromeliae* was a homonym, *Pseudococcus brevipes* was considered valid for several years (Zimmermann 1948). Ferris (1950) established a new genus *Dysmicoccus* and designated *Dactylopius brevipes* Cockerell as the type species in 1950.

Carter (1933b) noted a pink and gray dimorphism within mealybug populations, and varying disease symptoms associated with each color morph. Although the feeding of either color morph would result in chlorotic striping and wilt, the gray morph also

produced green spotting on plants while the pink morph did not (Carter 1933b). He referred to them as the “green spotting” and “non-green spotting” strains. Ito (1938) reported distinct differences in the biology of the color morphs. The pink strain was truly parthenogenetic whereas the gray had two distinct sexes and mating was absolutely necessary for reproduction. Differences in feeding habits of the two types were also noted. The gray type congregated mostly in the aerial plant parts producing the typical green spotting on leaves, whereas the pink type was found mostly among the roots, crown region and also in the aerial parts (Ito 1938; Gonzalez-Hernandez 1995). The microbial symbionts of the two ‘strains’ were also different, the green spotting strain exhibiting a rod shaped symbiont (Carter 1935). Based on distinct differences in morphological and biological characters, Beardsley (1959) declared the gray morph a new species that he named *Dysmicoccus neobrevipes* Beardsley, and retained the species name *D. brevipes* for the PPM. The systematics of *Dysmicoccus brevipes* has become more complex with the discovery of bisexual populations producing green spotting symptoms, but morphologically indistinguishable from *D. brevipes* in tropical America, Africa, and Malaysia (Lim 1972; Beardsley 1978; Beardsley 1993).

*Biology.* Ito (1938) studied the biology of PPM and GPM in Hawaii at ambient laboratory temperatures (ca. 23.5°C). PPM reproduced parthenogenetically. Eggs hatched within the female body, and a mean of  $234 \pm 13.4$  progeny were produced by a single female. There were three instars. Immatures spent a mean of 14.0, 9.8, and 10.3 days as first, second, and third instars, respectively, and required about 34 days to reach the adult stage. He also reported a pre-larvipositional period of 26.6 days, a larvipositional period of 24.8 days, and a post-larvipositional period of 4.7 days in the

adult stage. He verified that GPM reproduced sexually and produced a mean of  $346 \pm 9.39$  progeny. Female adults lived for 94.5 days. Ghose (1983) reported a much shorter life span for PPM in India, with the nymphal stage being 19 days long followed by a preovipositional period of 16 days, and an ovipositional period of 40 days when reared on potato at 30°C. PPM produced a mean of 240 progeny per female. Ghose (1983) reported that the crawlers had only two tail processes, the second instars had six pairs of processes, and the third instars and adults had 17 pairs of processes.

PPM is active in pineapple throughout the year, but its populations increase from December to June along with the increase in temperature (Lee and Chien 1967). A study conducted in Brazil showed that high infestations were favored by low humidity and high temperatures, but rainfall was detrimental (Santa Cecilia *et al.* 1992).

*Distribution and host range.* PPM is the most widely distributed tropicopolitan mealybug species throughout the tropical and subtropical parts of the world infesting more than 140 host plants (Williams and Watson 1988; Williams and Willink 1992; Ben Dov 1994). GPM was reported from Central and South America, some Pacific islands and a few locations in South Asia (Williams and Willink 1992), infesting more than 50 plant species (Williams and Watson 1988; Williams and Willink 1992; Ben Dov 1994). PPM is more commonly reported in the literature than GPM, even in South America where it is believed these mealybugs evolved.

Common host plants reported in Hawaii include guinea grass, *Panicum maximum* Jacq. (Poaceae), natal grass, *Rhynchelytrum repens* (Willd.) Hubb. (= *Tricholaena repens*) (Poaceae), celery, *Apium graveolens* L. (Apiceae), and taro, *Colocasia esculenta* (L.) Schott (Araceae) for PPM, and sisal, *Agave sisalana* Perrine (Agavaceae), *Portulaca*

sp. L. (Portulacaceae) and rhodesgrass, *Chloris gayana* Kunth (Poaceae), for GPM (Carter 1933b, 1951). The report of GPM from rhodesgrass is confusing because most of the subsequent literature reported rhodesgrass as a host of PPM and not GPM. Beardsley (1993) concluded that PPM commonly infested perennial grasses, including sugarcane, and GPM was abundant on sisal, but never infested perennial grasses. Ben Dov (1994) compiled a detailed list of host plants along with the world distribution of mealybugs and only listed *Zea mays* as a graminaceous host plant for GPM, whereas many plants including rhodesgrass were listed as hosts for PPM. In contrast, banana was infested by both species (Beardsley 1993). Rhodesgrass, red top natal and sand burr that are common in pastures and roadsides (Wagner *et al.* 1999), were considered as a reservoir for *Dysmicoccus* mealybugs on pineapple (Carter 1932a; Rohrbach *et al.* 1988). Whether mealybugs found on weeds serve as hosts for their parasitoid natural enemies is unknown.

### **Pineapple Mealybug Wilt Associated Virus and Etiology of MWP**

The etiology of MWP is not yet fully understood (Reimer and Beardsley 1990; German *et al.* 1992; Hughes and Samita 1998; Sether *et al.* 1998). Initially thought to result from mealybug feeding (Illingworth 1931a), MWP was suspected to be caused by mealybug-produced toxins (Carter 1933a; Carter 1937a, 1939). After Carter (1951) noted the failure of mealybugs to produce wilt symptoms without previously feeding on a 'positive' source, he suspected the involvement of a latent infection produced by a mealybug transmitted virus (Carter 1951, 1963, 1973).

Double-stranded virus particles were isolated from infected pineapple tissues from Hawaii, Australia and Cuba (Gunashinghe and German 1986). The virus was



assigned to the Closterovirus group, based on particle morphology and nucleic acid composition (Gunasinghe and German 1989). The virus initially called Pineapple Closterovirus (PCV) (Hu *et al.* 1996), has now been designated as Pineapple Mealybug Wilt Associated Virus (PMWaV) (Sether *et al.* 1998). Monoclonal antibodies suggested the existence of at least two distinct forms (PMWaV-1 and PMWaV-2) of PMWaV; both forms are widely distributed around the world (Sether *et al.* 2001), and both were detected from symptomatic as well as asymptomatic pineapple plants. PMWaV-1 was found from all the symptomatic plants tested in India, Costa Rica and Honduras, but none of the symptomatic plants from Malaysia had PMWaV-1 (Sether *et al.* 2001). MWP symptoms had a 100% correlation with PMWaV-2 in the plant samples collected from Hawaii and Malaysia, but not with PMWaV-1 (Sether *et al.* 2001), thereby supporting a long hypothesized virus association in the disease complex. Because many symptomless plants also tested positive to virus infection, it was concluded that the virus alone was not adequate for disease expression (Hu *et al.* 1996; Hu *et al.* 1997; Hughes and Samita 1998). A new genus, '*Ampelovirus*', has been proposed to include the mealybug-transmitted viruses, including PMWaV within the family Closteriviridae (Sether 2002).

Although German *et al.* (1992) reported the presence of virus from *Paspalum urvillei*, a common weed found around pineapple fields, and suspected the presence of 'severe strains' of viruses in other weeds, none of the plants growing near or in pineapple fields were identified as a host for PMWaV-1 (Sether 2002). Similarly, the virus was not detected in pineapple seedlings grown from seeds (Hu *et al.* 1996).

Insect transmission studies showed that both PPM and GPM could acquire and transmit PMWaV from diseased plants to healthy plants (Sether *et al.* 1998). The virus

was transmitted in a semipersistent manner, but was not transmitted transovarially. The virus was more efficiently transmitted by third instar mealybugs than earlier nymphal stages, and transmission declined with the aging of the adult mealybugs (Sether *et al.* 1998). Virus was detected in the mealybugs that fed on diseased pineapple plants, but not in mealybugs cultured on squash (Hu *et al.* 1996). Similar results were reported by (Carter 1952), indicating it was necessary for PPM / GPM to feed on a positive wilt source before they could transmit the disease. The same study also showed that the mealybugs would lose the capability to vector wilt if they passed through an intermediate host (e.g., agar plate in this case) before feeding on pineapple plants. PMWaV is phloem limited and not mechanically transmissible (Hu *et al.* 1997). The only other mode of dispersal for this virus (in addition to vector transmission) is through the vegetative propagation of pineapples through crowns and suckers (German *et al.* 1992; Sether 2002)

### **Ants and MWP**

The mutualistic relationships of ants and homopteran insects have been widely discussed (Nixon 1951; Buckley 1987; Holldobler and Wilson 1990; Huebner and Volkl 1996; Itioka and Inoue 1999). The major benefit of this association for ants is access to a renewable food source (i.e., honeydew), while for homopteran insects the benefits are: 1) protection from predators and parasitoids; 2) removal of honeydew which decreases physical fouling and incidence of fungal attack; 3) removal of dead individuals; and 4) construction of protective shelters (Buckley 1987).

Of the 40 ant species found in Hawaii, the big headed ant, Argentine ant, and fire ant are the most common and important ant species (Reimer 1994). *Pheidole megacephala* is aggressive and the most abundant ant species found in pineapple fields.

It was identified as a major factor in the survival and dissemination of *Dysmicoccus* mealybugs (Illingworth 1931a; Carter 1932a). These ants tend *Dysmicoccus* mealybugs for their honeydew, which is rich in glucose, amino acids and vitamins (Nixon 1951; Gray 1952). *Dysmicoccus* mealybugs rarely attain serious pest status in the absence of ants (Illingworth 1931a). Even artificial infestations of these mealybugs do not establish if ants do not attend them immediately (Carter 1937a). Early research on pineapple wilt credited ants with protecting *Dysmicoccus* mealybugs against their natural enemies by: 1) safeguarding and constructing mud shelters covering the mealybugs; 2) removal of honeydew; and 3) redistribution of mealybugs in the field (Illingworth 1931a; Carter 1932a; Collins 1960; McEwen *et al.* 1976). A high correlation between the presence of ants and mealybug incidence (Beardsley *et al.* 1982) and a decline of mealybug populations following ant control (Rai and Sinha 1980) support claims that mealybugs benefit from the presence of ants.

Both ants and mealybugs invade newly planted pineapple crops from surrounding field borders, and it takes about three years for the establishment of widespread populations of ants and mealybugs in a new planting (Beardsley *et al.* 1982). However, a laboratory study with GPM and big headed ant neither supported the benefits of honeydew removal nor the ants' role as a dispersing agent (Jahn 1992).

Later studies conducted in the laboratory and field provided further evidence of the direct protection that big headed ants provide to PPM (Gonzalez-Hernandez *et al.* 1999a). Although big headed ants do not kill the PPM parasitoid *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae), they greatly reduce the parasitoid's effectiveness in attacking PPM. The time spent in searching for PPM was not affected by the presence of

ants, but the total time that *A. ananatis* was in actual contact with mealybug individuals was 50 percent less in the presence of ants than in their absence (H. Gonzalez-Hernandez, unpublished data). This 50 percent reduction in contact time resulted in 61 percent reduction in PPM attack rate in the presence of ants. Laboratory studies showed that big headed ants feed upon the eggs, larvae, and adult stages of the coccinellid *Nephus bilucernarius* Muslant (Gonzalez-Hernandez 1995). Its predation rate was reduced as much as 70 percent in the presence of ants. Similar interference of natural enemy behavior by attending ants has been reported for other mealybugs and scale insects (Greathead 1978; DeBach 1980; Reyd *et al.* 1992; Cudjoe *et al.* 1993; Reimer *et al.* 1993).

### **Pineapple Mealybug Wilt Control**

Several approaches have been employed for control of PPM and GPM and the associated MWP. These include cultural, chemical, physical and biological control as well as host plant resistance. Some tactics are directed at the mealybugs while others focus on the attending ants or the closterovirus pathogen.

*Cultural controls.* Illingworth (1931a) suggested a thorough removal of mealybug infested plant debris and alternative weed hosts in old fields before planting a new crop, burning of plant residues to destroy the mealybugs, and screening of planting slips for green spotting symptoms. Emphasis was mainly on cultural practices such as maintaining general cleanliness around the field. Use of sunken boards as ant fences between the pineapple and weed borders and application of repellent on these fences were also practiced (Johnson 1935). Wilt incidence in the field moved faster when pineapple rows ended at an infested edge than when they were planted parallel to the

infested edge (Carter 1932b; Johnson 1935). A ‘guard row’ of three to six rows of pineapple was used to encircle the main part of the field. The guard rows were sprayed with oil emulsion to suppress MWP (Carter 1932b; Johnson 1935).

*Resistant varieties.* Selection of pineapple varieties that are resistant or tolerant to MWP was among the major goals of Hawaii-based pineapple-breeding programs. Early studies showed some encouraging results, but all selected lines succumbed to heavy mealybug infestations on further testing (Carter and Collins 1947; Collins and Carter 1954). Later, breeding for MWP resistance was dropped because ant control effectively reduced mealybug populations (and therefore MWP) in pineapple plantations (Williams and Fleisch 1993). The highly commercialized pineapple variety ‘smooth cayenne’ is highly susceptible to mealybugs and MWP. Utilization of chimeric transgenes from multiple viruses to develop broad spectrum resistance to PMWaVs in pineapple has been suggested (Sether 2002). However, even if resistance to MWP is achieved, *Dysmicoccus* mealybugs will still have to be controlled to limit fruit contamination and yield reductions due to mealybug feeding. Perhaps a better strategy would be to select for resistance to the mealybug vectors and not the MWP.

*Physical controls.* Ullman *et al.* (1991) reported that the use of a hot water treatment for pineapple suckers before planting was useful for increasing yield. Suckers were submerged in large baths at 40°C for one hour or 50°C for one half-hour. Heat-treated plants grew faster and produced more vigorous plants than non-treated plants, and the associated closterovirus was not detected throughout the plants’ growth (Ullman *et al.* 1993).

*Chemical controls.* Before the development of synthetic organochlorine insecticides, mixtures of various soaps, tobacco extract, mercury, sulphur, and various oils were recommended as plant dips and / or sprays to control scales and mealybugs infesting pineapple (Rohrbach *et al.* 1988). After the development of synthetic pesticides, organochlorine and organophosphate insecticides provided excellent mealybug and ant control (Huang and Chien 1969; Menezes *et al.* 1977; Rohrbach *et al.* 1988; Santa Cecilia L. V *et al.* 1992; Sanches *et al.* 1995). Fumigation of planting materials with methyl bromide was found to be an effective mealybug control in South Africa (Petty 1987).

Organochlorine insecticides such as mirex, chlordane and heptachlor effectively controlled the tending ants until their registrations were canceled during the 1980's due to their negative impacts on the environment (Rohrbach *et al.* 1988). No effective formicides are currently registered for use in pineapple (Reimer *et al.* 1990). Presently, ant control is achieved by bait applications of hydramethylnon (Amdro<sup>®</sup>) (Reimer and Beardsley 1990) with annual renewal of a emergency needs registration (Section 18). If the emergency petition for Amdro<sup>®</sup> were refused, the Hawaiian pineapple industry would greatly suffer because no effective management tactics exist that do not require pesticides for mealybug control (Gonzalez-Hernandez 1995).

*Biological control.* A biological control program specifically targeting pineapple-infesting mealybugs in Hawaii began in 1922 with the introduction of several coccinellids from Panama (Canal Zone) (Swezey 1925). Foreign explorations in North, Central and South America, as well as Asia and Africa in the 1930's and later led to the

introduction of more than 20 predators and parasitoids (Tables 2.1 and 2.2) (Sakimura 1939; Funasaki *et al.* 1988; Gonzalez-Hernandez 1995). The last introduction of *Dysmicoccus* natural enemies in Hawaii occurred in 1954 (Weber 1955), several years prior to the identification of GPM as a distinct species (Beardsley 1959). A few introductions were also made from South America, a hypothesized site of mealybug origin (Gonzalez-Hernandez 1995).

A recent survey by Gonzalez-Hernandez *et al.* (1999b) on the Hawaiian islands of Oahu and Maui revealed the presence of only five *Dysmicoccus* natural enemies: *Anagyrus ananatis*, *Euryrhopalus propinquus* Kerrich (Hymenoptera: Encyrtidae), *Nephus bilucernarius* Mulsant (Coleoptera: Coccinellidae), *Sticholotis ruficeps* Weise (Coleoptera: Coccinellidae), and *Lobodiplosis pseudococci* (Felt) (Diptera: Ceceidomyiidae). Survey results showed that *A. ananatis* was the most common biological control agent in pineapple plantings, followed by *N. bilucernarius*. The level of parasitization by the endoparasitoid *E. propinquus*, which primarily attacks GPM, ranged from 0.2 to 1.7 percent (Gonzalez-Hernandez 1995). However, a prior field survey failed to recover any biological control agent of the gray pineapple mealybug (Jahn 1992).

### **Biological Control**

*Definition and categories.* In the simplest terms, biological control of arthropods is the utilization of parasitoids, predators and pathogens for the regulation of pest populations (DeBach 1964a). Although there are continuous attempts to broaden the scope of biological control by including biologically-based pest management methods (e.g., sterile insect techniques, host plant

Table 2. 1. Parasitoids introduced to Hawaii to control *Dysmicoccus* mealybugs  
(after Gonzalez-Hernandez 1995).

Year	Parasitoid	Origin	Reference
1924	Chalcid*	Panama Canal Zone	Swezey 1925
1932	<i>Zaplatycerus fullawayi</i> Timberlake	Panama	Sakimura 1939
1935	<i>Synaspidia pretiosa</i> (Timberlake)	Guatemala	Sakimura 1939
1935	<i>Aenasius cariococcus</i> Compere	Colombia	Chapman 1938
1935	<i>Aenasius colombiensis</i> Compere	Colombia	Sakimura 1939
1935-36	<i>A. colombiensis</i>	Colombia, Venezuela	
1935-37	<i>Anagyrus ananatis</i> Gahan	Brazil	Sakimura 1939, Carter 1937
1935-37	<i>Hambletonia pseudococcina</i> Compere	Brazil, Venezuela, Colombia	Sakimura 1939
1936	<i>Euryrhopalus propinquus</i> Kerrich	British Guyana	Funasaki <i>et al.</i> 1988
1938	<i>Anagyrus aurantifrons</i> Compere	Africa	Chapman 1938
1954	<i>Pseudophycus angelicus</i> (Howard)	California	Webber 1955
?	<i>Coelaspida osborni</i> Timberlake	?	Noyes and Hayat 1994
?	<i>Leptomastidea abnormis</i> (Girault)	?	Noyes and Hayat 1994

\*All the parasitoids belong to Hymenoptera: Encyrtidae, except the chalcid imported in 1924.



Table 2. 2. Predators introduced to Hawaii for the control of *Dysmicoccus* mealybugs  
(after Gonzalez-Hernandez 1995).

Year	Predator	Origin	Reference
<u>Cecidomyiidae</u>			
1930	<i>Lobodiplosis pseudococci</i> Felt.	Mexico	Sakimura 1939
<u>Coccinellidae</u>			
1922	<i>Diomus</i> sp.	Mexico	Swezey 1925
	<i>Diomus</i> sp 2	Mexico	Swezey 1925
	<i>Hyperasis sylvestrii</i> Weise	Mexico	Swezey 1925
	<i>Nephus</i> sp.	Mexico	Swezey 1925
	<i>Scymnus (Pullus) uncinatus</i> Sicard	Mexico	Funasaki <i>et al.</i> 1988
1924	<i>Hyperasis albicollis</i> Gorh.	Panama Canal zone	Swezey 1925
	<i>Cleothera bromelicola</i> Sic.	Panama Canal zone	Swezey 1925
	<i>Diomus margipallens</i>	Panama Canal zone	Swezey 1925
	<i>Scymnus pictus</i> Gorh.	Panama	Swezey 1925
	<i>S. (Pullus) uncinatus</i>	Panama	Swezey 1925
1930	<i>Nephus bilucernarius</i> Mulsant	Mexico	Swezey 1925
1933	<i>Diomus futahoshii</i> Ohta	Formosa	Chapman 1938
	<i>Cryptolaemus montrouzieri</i> Mulsant	California	Sakimura 1939

Continued on next page

Table 2. 3. (Continued) Predators introduced to Hawaii for the control of *Dysmicoccus* mealybugs  
(after Gonzalez-Hernandez 1995).

Year	Predator	Origin	Reference
1935	<i>Hesperasis c-nigrum</i> Mulsant	Brazil	Sakimura 1939
1936	<i>Hyperasis quinquenotata</i> Mulsant	Brazil	Sakimura 1939
	Coccinellid sp.	Brazil	Sakimura 1939
1937	<i>Hyperasis</i> sp.	Brazil	Sakimura 1939
	<i>Diomus</i> sp.	Brazil	Sakimura 1939
1938	<i>Scymnus apiciflavus</i> Motsch.	Malaysia	Sakimura 1939
<u>Drosophilidae</u>			
1924	<i>Pseudiastata nebulosa</i> Coquillett	Mexico	Swezey 1925
1931-1937	<i>Pseudiastata nebulosa</i> Coquillett	Panama, British Guyana, Guatemala, Trinidad	Sakimura 1939
<u>Hemerobiidae</u>			
1929	<i>Sympherobius barberi</i> Banks	Mexico	Swezey 1925

resistance, insect pheromones, and genetic manipulation of pest & plant species), workers in the field still limit the definition to situations in which a definite interaction occurs between a target species and its natural enemies (DeBach and Rosen 1991). Modern biological control programs are broadly divided into three major categories: classical (introduction), augmentation and conservation (DeBach 1964a; van Driesche and Bellows 1996).

*Classical biological control.* In a given environment, organisms tend to maintain more or less consistent relationships with other species which may benefit or impair their existence (Doutt and DeBach 1964). Predator-prey and parasitoid-host relationships are common and contribute towards limiting population densities of many species, including arthropod pests. Pest numbers can increase enormously when they enter a new geographical location and thereby escape the suppression exerted by natural enemies in their home range (Doutt and DeBach 1964). With an increase in international trade, the risk of movement of non-indigenous arthropod pests has increased dramatically (Jenkins 1996; McNeely 2000).

The introduction of effective natural enemies from a pest's native home is the traditional way of implementing biological control (Doutt and DeBach 1964). The first, and one of the most successful, modern biological control programs was implemented in California to control the cottony cushion scale, *Icerya purchasii* Maskell (Homoptera: Margarodidae), by importing its natural enemies, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) and *Cryptochetum iceryae* (Williston) (Diptera: Cryptochetidae) from Australia. Encouraged by the success of early biological control projects, Hawaii became one of the prominent centers of biological control studies

(DeBach and Rosen 1991). Over 500 insects have been targeted worldwide in more than 1200 biological control introduction programs, resulting in complete or partial control of more than 200 insect species (van Driesche and Bellows 1996). Recently, some concerns have been raised about the non-target impacts of introduced organisms (Bentley and O'Neil 1997). The egg parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) which was successfully applied to suppress corn stem borer *Ostrinia nubilalis* in Europe, had minimal impact on non target lepidopterous hosts under natural environment despite their susceptibility under laboratory conditions (Bigler *et al.* 2002). Similarly, the imported fruit fly parasitoids *Tetrastichus giffardianus* Sylvestry (Hymenoptera: Eulophidae) and *Dichosmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) had minimal impact on natural populations of endemic fruit fly in Hawaii although the parasitoids successfully attacked and completed their lifecycle in these hosts when the flowerhead galls were dissected and maggots presented to the parasitoids (Duan *et al.* 1997; Duan and Messing 1998). On the other hand, *Compsilura concinnata* (Meigan) (Diptera: Tachinidae), that has been introduced repeatedly to North America from 1906 to 1986 as a biological control agent against several pest species attacked many silk moth including *Hemileuca maia maia* (Lepidoptera: Saturnidae), a state-listed threatened species of New England (USA) (Boettner *et al.* 2000). Studies conducted to evaluate the environmental risks of biological control agents in Europe suggested that about 10% or less of classical biological control introductions and 49% of inundative or augmentative uses of agents led to (local, short- term) population changes for non-targets (Lynch *et al.* 2001).

*Augmentation.* Augmentation deals with the manipulation of natural enemies to make them more efficient. This may be achieved by periodic, repetitive colonization (sometimes combined with their hosts) or genetic improvement for desired characteristics (e.g., increased host searching abilities, resistance to pesticides, enhanced developmental rate, increased temperature tolerance or by employing chemical cues to affect their behavior) (Huffaker *et al.* 1977; van Driesche and Bellows 1996). Augmentation is practiced when an environment either lacks natural enemies (they cannot survive through extreme environmental conditions) as in European and Canadian greenhouses (Parella *et al.* 1999) or arrives late or in too few numbers to provide adequate pest control (McKenzie 1967; van Driesche and Bellows 1996). Augmentative releases can overcome these constraints and enhance the build up of natural enemies by reducing the time lag in the numerical response of predator/parasite populations (Huffaker *et al.* 1977).

A survey conducted in Florida revealed that insects are the most common targets of augmentative biological control. Homopteran insects had the largest share (41%) of targeted cases (Frank and McCoy 1994). The most common augmentative biological agent used against insects is the pathogen *Bacillus thuringiensis* Berliner which mostly controls lepidopterous pests (DeBach and Rosen 1991). Worldwide use of *Trichogramma* spp. (egg parasitoids of lepidopterous pests), *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (predator of spider mites) and *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (parasitoid of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) are among the most common examples of augmentative biological control using arthropod natural enemies (DeBach and Rosen 1991; Elzen and King 1999). Numerous commercial insectaries produce a

variety of biological control agents such as *Trichogramma*, green lacewings, lady beetles, predatory mites and other parasitoids in the USA (DeBach and Rosen 1991).

Augmentative releases of entomophages are classified into: 1) inoculative releases (i.e., small numbers of natural enemies are released and control is achieved by their offspring for considerably long periods of time); and 2) inundation (i.e., pest control is achieved directly by the action of the organisms released in large numbers) (DeBach and Hagen 1964; Knipling 1977; DeBach and Rosen 1991; Elzen and King 1999).

Augmentation is generally considered an environmentally sound, viable alternative to chemicals, which may also create local employment (Bentley and O'Neil 1997). The major limitation for the successful application of augmentative biological control is the lack of cost effective rearing methods for biological control agents (Morrison and King 1977; Gross 1994; Elzen and King 1999). Quality is also a major concern related to mass reared organisms. Quality is associated with the fitness and adaptability of the organism to environmental conditions, its host searching capacity, and the power to increase in numbers relative to its host (Huffaker *et al.* 1977). A poor quality agent could result in failure to achieve biological control (Knipling 1977).

*Conservation.* Whether introduced, augmented or naturally occurring, natural enemies have to survive and effectively work at the site of release. Thus, conservation of the natural enemies becomes critical. According to DeBach and Rosen (1991), the greatest possibility of utilization of biological control agents lies in their conservation. Conservation can be achieved by manipulating the environment or agronomic practices to make a habitat more favorable to natural enemies (DeBach 1964a). The most common examples of conservation of biological control agents are the selection and modification

of pesticides and their application methods; as well as modification of crop husbandry practices to provide better shelter and food for natural enemies (DeBach, 1964; van Driesche and Bellows 1996; Elzen and King 1999). Conservation allows farmers to preserve native species, save labor and money, and reduce pesticide applications (Bentley and O'Neil 1997).

### **Biological Control of Mealybugs**

Coccids (i.e., scales and mealybugs) are among the most easily transported insects that can establish to become serious pests of economically important crops (DeBach 1964b, 1980). Many pestiferous mealybugs (Homoptera: Pseudococcidae), including *Dysmicoccus* spp., have been targeted for biological control (DeBach and Rosen 1991). Some of the most outstanding successes in biological control were with mealybugs.

Citrophilus mealybug, *Pseudococcus calceolariae* (Maskell), a presumed native of Australia, was first reported in California in 1913. This mealybug was a severe pest of citrus and other ornamental crops in California, New Zealand, and The Crimea. Two parasitoids, *Tetracnemoidea brevicornis* (Girault) and *Coccophagus gurneyi* Compere (Hymenoptera: Aphelinidae), and several predators were imported from Australia to California. The parasitoids readily established and provided adequate control of the mealybug within a year. *Coccophagus gurneyi* was later introduced to Russia and Chile, where it provided equally good control of the mealybug (DeBach and Rosen 1991).

Citriculus mealybug, *Pseudococcus citriculus* Green, was first noticed in Israel in 1938, with severe outbreaks in some coastal plains. Of the three parasitoids imported in 1940 from Japan, *Clausenia purpurea* Ishii readily reproduced in this mealybug and

apparently suppressed populations to below detectable levels (Bartlett 1978; DeBach and Rosen 1991)

Rhodesgrass mealybug, *Antonina graminis* (Maskell), is unique among mealybugs because it loses its legs and remains sessile after the first instar. It is distributed throughout many Asian countries and Pacific islands. The mealybug attacks grass species including rhodesgrass, Bermuda grass, Johnson grass, sugarcane and St. Augustine grass. After its discovery in 1942, *A. graminis* was considered a serious pest in Texas and Mexico. *Anagyrus antoniana*e Timberlake was introduced from Hawaii and provided a high level of control in cool and humid areas, but failed to establish in hot arid regions. *Neodusmetia sangwani* (Subba Rao) was later introduced from India in 1959 and readily established in the hot arid areas, providing adequate control of the mealybug (Bartlett 1978; DeBach and Rosen 1991).

Coffee mealybug, *Planococcus kenyae* (LePelley), spread rapidly in the highland coffee plantings of Kenya following its initial discovery in 1923. Initial misidentification led to the importation of parasitoids that would not breed on coffee mealybug. After the introduction of five encyrtid species from Uganda in 1938, *Anagyrus* sp. nr. *kivuensis* Compere (Hymenoptera: Encyrtidae) provided the most effective suppression of the mealybug populations within 6-8 months (Bartlett 1978; DeBach and Rosen 1991).

A recent example of a highly successful program was the control of cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, in Africa. This mealybug was first discovered in West Africa in 1974 and described as a new mealybug species. Research studies conducted by the International Institute of Biological Control (IIBC), International Institute of Tropical Agriculture (IITA), and Centro Internacional de



Agricultura Tropical (CIAT) with the support of several national and international agencies helped in the discovery of the encyrtid *Apoanagyrus* (= *Epidinocarsis*) *lopezi* De Santis, in South America. Introduction of *A. lopezi* to Africa in the 1980's finally brought the pest under control. (Neuenschwander and Hammond 1988; Neuenschwander 1996)

The mango mealybug, *Rastrococcus invadens* Williams, was accidentally introduced to Africa in 1980-81 and it became a pest on various fruit trees. *Gyranusoidea tebygi* Noyes and *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) were introduced from India and were readily established. They quickly suppressed the mealybug population (Neuenschwander *et al.* 1994; Bokonon-Ganta and Neuenschwander 1995; Bokonon-Ganta *et al.* 1995; Bokonon-Ganta *et al.* 1996; Bokonon-Ganta *et al.* 1997)

Pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), reportedly a native of India, became one of the most injurious pests of many crops in Egypt following its introduction around 1908. Importation and periodic releases of *Cryptolaemus montrouzieri* were of little help, but introduction of *Anagyrus kamali* Moursi and *Achrysoptophagus* had outstanding effects (Bartlett 1978). Following its introduction to Grenada in 1994, this mealybug invaded and established in South America and the Caribbean Basin (Sagarra and Peterkin 1999; Kairo *et al.* 2000). Introduction of the parasitoid, *Anagyrus kamali*, from China through the International Institute of Biological control (IIBC) has shown promising results (Sagarra and Peterkin 1999). This mealybug was accidentally introduced into the Imperial Valley of California in 1999. Introduction of *A. kamali* within one month of the first detection of the mealybug in California was

possible due to the preemptive thrust provided by the USDA, following the development of a biological control program in the Caribbean. The mealybug is now effectively controlled and restricted to Imperial County in California (D. Meyerdirk, Personal communication). Comparable success has been achieved for the control of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Homopteran: Pseudococcidae), in Florida, the Bahamas and most recently in Chicago, Illinois, with the introduction of four exotic parasites (*Anagyrus loecki* Noyes and Menezes, *Pseudaphycus angelicus* (Howard), *Apoanagyrus californicus* Compere and *Acerophagus papayae* Noyes and Schauff) (D. Meyerdirk, Personal communication).

PPM and GPM were reported from Hawaii in 1910 as *Pseudococcus brevipes*. Several biological control agents were imported, of which five established. The encyrtid parasitoids *Anagyrus ananatis*, *Euryrhopalus propinquus* and *Hambletonia pseudococcina* and predators such as *Nephus bilucernarius*, *Sticholotis pseudococci* and *Lobodiplosis pseudococcina* do not provide adequate control of these mealybugs when ants are present. Farmers largely rely on foliar applications of diazinon or bait applications of andro to kill tending ants for the control of the mealybugs (Carter 1944; Rohrbach *et al.* 1988; Gonzalez-Hernandez 1995; HDOA 2001).

Augmentative releases of *Anagyrus punctulatus* Agarwal against sugarcane mealybug, *Saccharicoccus sacchari* (Cockerell), in India provided promising results (Kapadia *et al.* 1995). Periodic releases of a green lacewing, *Chrysoperla rufilabris* (Burmeister), reduced populations of the long tailed mealybug below aesthetic injury levels for four weeks (Goolsby *et al.* 2000). Populations of the grape mealybug,

*Maconellicoccus hirsutus* (Green), were suppressed by augmentative releases of adults or larvae of *Cryptolaemus montrouzieri* in grapes in India (Babu and Azam 1989).

### **Factors Affecting Augmentative Biological Control**

Utilization of any parasitoid or predator in augmentative biological control is constrained mainly by the ability to economically mass produce the entomophage in captivity (Morrison and King 1977). However, ease of mass production should not be the only criterion used to choose biological control agents (DeBach and Rosen 1991). The main objective of culturing biological control agents is to provide a condition that yields an abundance of optimum quality females for release (Luck *et al.* 1999). Natural enemies used for augmentation must be able to recognize, locate, successfully attack and reproduce in the target pest (van Driesche and Bellows 1996). Fitness and adaptability to the host and its environment, searching capacity, power of increase relative to the host or prey, and host / prey specificity are other important characteristics to be considered (Huffaker *et al.* 1977). Failure of a biological control agent to suppress the pest may be related to inadequate numbers or improper application of techniques rather than the inability of the agent used (Knipling 1977). It is therefore important to produce high quality parasitoids in the laboratory to achieve a good level of field activity. Some of the measurable traits used to express the quality of mass produced insects include sex ratio, emergence rate, body size, head width, and hind tibia length. Insect size alone may not be a good measure of the quality, but it is often related to many other quality parameters of parasitoids (Etzel and Legner 1999). The size of a laboratory-reared organism is an important determinant of quality because it influences fecundity and host finding (Luck *et*

*al.* 1999). Reduction in the size of a laboratory-reared parasitoid may lead to a reduction in its fecundity and longevity (Waage *et al.* 1985).

### **Biological Control Approaches for *Dysmicoccus brevipes***

Biological control of PPM in Hawaii has focused on the introduction of natural enemies from its native home. Some of the imported biological control agents have successfully established, while others have not (Gonzalez-Hernandez *et al.* 1999b). This strategy has produced mixed results. The attempt at controlling *D. brevipes* with the introduction of *C. montrouzeri* in Africa failed, but partial success has been achieved in Puerto Rico with the introduction of *A. ananatis* and *H. pseudococcina*.

Failure of biological control agents to suppress PPM has been attributed to the associated ants, especially the big-headed ant. (Rohrbach *et al.* 1988; Jahn and Beardsley 1998) In contrast, Carter (1945) reported complete control of pineapple mealybugs by natural enemies (in an abandoned pineapple crop in Hawaii) without ant control. The longer time required to achieve mealybug control under such circumstances may be attributed to ant interference leading to slower natural enemy colonization as well as lower parasitization rates. Bait applications of Amdro® to eliminate ants conserves the natural enemies and also enhances their activities. Augmentation of natural enemies by releasing laboratory-reared parasitoids to increase the ratio of natural enemies to the attending ants, thereby increasing the probability of mealybug biological control in the presence of ants, has been suggested (Gonzalez-Hernandez 1995), but never been implemented.

*Anagyrus ananatis*: A candidate for augmentation. A large number of encyrtid wasps are used in classical biological control programs worldwide due to their close

association with Homoptera, especially the coccids (Clausen 1940; Noyes and Hayat 1994). *Anagyrus* spp., primary endoparasitoids of mealybugs, are the most widely used anagryine wasps in biological control programs (Noyes and Hayat 1994). Numerous encyrtids have been introduced to Hawaii to control scales and mealybugs (Beardsley 1978). *Anagyrus ananatis*, the most common parasitoid of pineapple mealybug in South America (Carter 1949), was introduced from Brazil in 1935 for the control of *Dysmicoccus* mealybugs in Hawaiian pineapple (Carter 1937b). PPM is the only known host of *A. ananatis* in Hawaii (Noyes and Hayat 1994), although DeSantis recorded it from *Antonina graminis* (Maskell), *Ferrisia virgata* (Cockerell), and *Planococcus citri* (Risso) in South America (Noyes and Hayat 1994).

*Anagyrus ananatis* was the most common parasitoid recovered from PPM tended by ants in pineapple plantations on Oahu and Maui (Gonzalez-Hernandez *et al.* 1999b). Based on its prevalence, host specificity and persistence, *A. ananatis* has been considered a good candidate for augmentative biological control of PPM (Gonzalez-Hernandez 1995). Gonzalez-Hernandez (1995) found that *A. ananatis* completes its life cycle in third instar and adult PPM (with preference for adults) in about 23 days at 25°C. Adult females survive for a mean of 10 days and lay about 27 eggs in their lifetime. Mass production techniques for *A. ananatis* have not yet been developed.

## CONCLUSIONS

Mealybugs are among the most successful invasive species that travel with plant materials and establish in new environments. Because of their cryptic habits, chemical control is difficult. Because they attack many economically important plant species

worldwide, classical biological control has become a common control strategy. In most cases, introduction of one or more natural enemies has provided adequate control of mealybugs. *Dysmicoccus* mealybugs have been the most difficult mealybug species to control. Their ability to vector plant pathogens that induce MWP results in their elevated pest status in pineapple.

Augmentative control has provided encouraging results with many mealybugs. Given a lack of numerous control alternatives, the potential of natural enemy augmentation should be tested. A potentially more rewarding tactic would be to look for additional natural enemies of *Dysmicoccus* mealybugs, especially GPM, because natural enemy introductions to Hawaii were terminated before the recognition of GPM.

## REFERENCES

- Abo, M. E. and A. A. Sy. 1998. Rice virus diseases: epidemiology and management strategies. *Journal of Sustainable Agriculture* 11: 2-3.
- Adu-Ampomah, Y., G. K. Owusu, S. Sackey, B. Padi and A. Abdul-Karimu. 1996. Use of gamma rays to induce mutants resistant to cocoa swollen shoot disease in *Theobroma cacao* L. *Plant Breeding* 115: 74-76.
- Babu, T. R. and K. M. Azam. 1989. Biological control of grape mealybug *Maconellicoccus hirsutus* (Green). *Indian Journal of Plant Protection* 17: 123-126.
- Bartlett, B. R. 1978. Pseudococcidae, pp. 137-171. *In* *Introduced Parasites and Predators of Arthropod Pest and Weeds: A World Review* (C. P. Clausen Ed.), U. S. Department of Agriculture, Handbook No. 480, Washington D.C.
- Beardsley, J. W. 1959. On the taxonomy of pineapple mealybugs in Hawaii, with the description of previously unnamed species (Homoptera: Pseudococcidae). *Proceedings of the Hawaiian Entomological Society* 17: 29-37.
- Beardsley, J. W. 1965. Notes on the pineapple mealybug in Hawaii with the description of two new species (Homoptera: Pseudococcidae). *Proceedings of the Hawaiian Entomological Society* 19: 55-68.
- Beardsley, J. W. 1978. Some thoughts on mealybug and mealybug management. pp. 65-69. *In* (K. F. Nwanje and K. Leuschner Ed.). *Proceedings, International Workshop on the Cassava Mealybug, P. manihotii* Mat-Ferr (Pseudococcidae), June 26-29, 1977 Bas-Zaire, Zaire.

- Beardsley, J. W., T. H. Su, F. L. McEwen and D. Gerling. 1982. Field investigations on the interrelationships of the big headed ant, the gray mealybug and the pineapple wilt disease in Hawaii. *Proceedings of the Hawaiian Entomological Society* 24: 51-67.
- Beardsley, J. W. 1993. The pineapple mealybug complex: taxonomy, distribution and host relationships. pp. 383-386. *In "Acta Horticulturae 334"* (D. P. Bartholomew and K. G. Rohrbach Ed.). *Proceedings, First International Pineapple Symposium*, 2-6 November, 1992 Honolulu, Hawaii.
- Ben Dov, Y. 1994. *A Systematic Catalogue of the Mealybugs of the World (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with Data on Geographical Distribution, Host Plants, Biology and Economic Importance*. Intercept, Andover, UK, 686 pp.
- Bentley, J. W. and R. J. O'Neil. 1997. On the ethics of biological control of insect pests. *Agriculture and Human Values* 14: 283-289.
- Bigler, F., D. Babendreier and S. Kuske. 2002. Ecological risks due to biological control of the European corn borer with *Trichogramma*. *Agrarforschung* 9: 316-321.
- Boettner, G. H., J. S. Elkinton and C. J. Boettner. 2000. Effects of a biological control introduction on three nontarget native species of Saturniid moths. *Conservation Biology* 14: 1798-1806.
- Bokonon-Ganta, A. H. and P. Neuenschwander. 1995. Impact of the biological control agent *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) on the mango mealybug, *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae), in Benin. *Biocontrol Science and Technology* 5: 95-107.



- Bokonon-Ganta, A. H., P. Neuenschwander, J. J. M. V. Alphen, M. Vos and J. J. M. van Alphen. 1995. Host stage selection and sex allocation by *Anagyrus mangicola* (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug, *Rastrococcus invadens* (Homoptera: Pseudococcidae). *Biological Control* 5: 479-486.
- Bokonon-Ganta, A. H., J. J. M. V. Alphen and P. Neuenschwander. 1996. Competition between *Gyranusoidea tebygi* and *Anagyrus mangicola*, parasitoids of the mango mealybug, *Rastrococcus invadens*: Interspecific host discrimination and larval competition. *Entomologia Experimentalis et Applicata* 79: 179-185.
- Bokonon-Ganta, A. H., J. J. M. V. Alphen, P. Neuenschwander and J. J. M. van Alphen. 1997. Competition between two parasitoids of the mango mealybug. *IITA Research* 14-15.
- Buckley, R. 1987. Interaction involving plants, homoptera and ants. *Annual Review of Ecology and Systematics* 18: 111-135.
- Carter, W. 1932a. Pineapple mealybug, *Pseudococcus brevipes* (Ckll), occurring on pineapple plants. *Ecology* 13: 296-304.
- Carter, W. 1932b. Border plantings as guard rows in pineapple mealybug control. *Journal of Economic Entomology* 25: 1027-1031.
- Carter, W. 1933a. The pineapple mealybug *Pseudococcus brevipes* and wilt of pineapple. *Phytopathology* 23: 207-242.
- Carter, W. 1933b. The spotting of pineapple leaves caused by *Pseudococcus brevipes*, the pineapple mealybug. *Phytopathology* 23: 243-259.
- Carter, W. 1934. Mealybug wilt and green spot in Jamaica and Central America. *Phytopathology* 24: 424-426.

- Carter, W. 1935a. Studies on biological control of *Pseudococcus brevipes* (Ckl.) in Jamaica and central America. *Journal of Economic Entomology* 28: 1037-1041.
- Carter, W. 1935b. The symbioants of *Pseudococcus brevipes* (Ckll.). *Annals of the Entomological Society of America* 28: 60-64.
- Carter, W. 1937a. The toxic dose of mealybug wilt of pineapple. *Phytopathology* 27: 971-981.
- Carter, W. 1937b. Importation and laboratory breeding of two chalcid parasites of *Pseudococcus brevipes* (Ckl.). *Journal of Economic Entomology* 30: 370-372.
- Carter, W. 1939. Injuries to plants caused by insect toxins. *Botanical Review* 5: 273-326.
- Carter, W. 1942. The geographical distribution of mealybug wilt with notes on some other insect pests of pineapple. *Journal of Economic Entomology* 35: 10-15.
- Carter, W. 1944. Biological control of *Pseudococcus brevipes* (Ckll.). *Proceedings of the Hawaiian Entomological Society* 12: 15.
- Carter, W. 1945. Encyrtid parasites of *Pseudococcus brevipes* (Cockerell). *Proceedings of the Hawaiian Entomological Society* 12: 489.
- Carter, W. and J. L. Collins. 1947. Resistance to mealybug wilt of pineapple with special reference to Cayenne-Queen hybrid. *Phytopathology* 37: 332-348.
- Carter, W. 1948. The effects of mealybug feeding on pineapple plants grown in finely atomized nutrient solutions. *Phytopathology* 38: 645-657.
- Carter, W. 1949. Insects notes from the South America with special reference to *Pseudococcus brevipes* and mealybug wilt. *Journal of Economic Entomology* 42: 761-766.

- Carter, W. 1951. The feeding sequence of *Pseudococcus brevipes* (Ckll) in relation to mealybug wilt of pineapple in Hawaii. *Phytopathology* 41: 769-780.
- Carter, W. 1952. Injuries to plants caused by insect toxins II. *Botanical Review* 680-687.
- Carter, W. 1963. Mealybug wilt of pineapple: A reappraisal. *Annals of the New York Academy of Sciences* 105: 741-764.
- Carter, W. 1973. *Insects in Relation to Plant Disease*. J. Willey and Sons, New York, 759 pp.
- Chapman, R. N. 1938. Biological control Project 26, pp 319-333. *In* Monthly report of the experimental Station of Pineapple Producers Cooperative Association.) Honolulu, HI.
- Clausen, C. P. 1940. *Entomophagous Insects*. McGraw Hill Book Co, New York, 688 pp.
- Collins, J. L. and W. Carter. 1954. Wilt resistant mutation in Cayenne variety of pineapple. *Phytopathology* 44: 662-666.
- Collins, J. L. 1960. *The Pineapple: Botany, Cultivation and Utilization*. Leonard Hill, London, 295 pp.
- Cudjoe, A. R., P. Neuenschwander and M. J. W. Copland. 1993. Interference by ants in biological control of the cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) in Ghana. *Bulletin of Entomological Research* 83: 15-22.
- DeBach, P. 1964a. The scope of biological control, pp. 3-20. *In* *Biological Control of Insects Pests and Weeds* (P. DeBach Ed.), Chapman and Hall, London.
- DeBach, P. 1964b. Successes, trends and future possibilities, pp. 673-713. *In* *Biological Control of Insect Pests and Weeds* (P. DeBach Ed.), Chapman and Hall, London.

- DeBach, P. and K. S. Hagen. 1964. Manipulation of Entomophagous species, pp. 429-458. *In* Biological Control of Insect Pests and Weeds (P. DeBach Ed.), Chapman and Hall, London.
- DeBach, P. and D. Rosen. 1991. Biological Control by Natural Enemies. Cambridge University Press, Cambridge, UK, 440 pp.
- Doutt, R. L. and P. DeBach. 1964. Some Biological control concepts and Questions, pp. 118-142. *In* Biological Control of Insect Pests and Weeds (P. DeBach Ed.), Chapman and Hall, London.
- Duan, J. J., M. Ahmad, K. Joshi and R. H. Messing. 1997. Evaluation of the impact of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) on a nontarget tephritid, *Eutreta xanthochaeta* (Diptera: Tephritidae). *Biological Control* 8: 58-64.
- Duan, J. J. and R. H. Messing. 1998. Effect of *Tetrastichus giffardianus* (Hymenoptera: Eulophidae) on nontarget flowerhead-feeding tephritids (Diptera: Tephritidae). *Environmental Entomology* 27: 1022-1028.
- Elzen, G. W. and E. G. King. 1999. Periodic release and manipulation of natural enemies, pp. 253-270. *In* Handbook of Biological Control: Principles and Applications of Biological Control (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.
- Etzel, L. K. and E. F. Legner. 1999. Culture and colonization, pp. 125-198. *In* Handbook of Biological Control: Principles and Applications of Biological Control (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.

- FAO. 2001. Production Yearbook. Food and Agriculture Organization of the United Nations, Rome, 235 pp.
- Ferris, G. F. 1950. Atlas of the Scale Insects of North America. Series V. The Pseudococcidae (Part 1). Stanford University Press, California, 1-278 pp.
- Frank, J. H. and E. D. McCoy. 1994. Commercial importation into Florida of invertebrate animals as biological control agents. Florida Entomologist 77: 1-20.
- Funasaki, G. Y., P. Y. Lai, L. M. Nakamura, J. W. Beardsley and A. K. Ota. 1988. A review of biological control introductions in Hawaii 1890-1985. Proceedings of the Hawaiian Entomological Society 28: 105-160.
- Garau, R., V. A. Prota, D. Boscia, M. Fiori and U. Prota. 1995. *Pseudococcus affinis* Mask., new vector of grapevine trichoviruses A and B. Vitis 34: 67-68.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. Advances in Disease Vector Research 9: 241-259.
- Ghose, S. K. 1983. Biology of parthenogenetic race of *Dysmicoccus brevipes* (Cockerell) (Pseudococcidae: Hemiptera). Indian Journal of Agricultural Sciences 53: 939-942.
- Gonzalez-Hernandez, H. 1995. The Status of Biological Control of Pineapple Mealybugs in Hawaii. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Gonzalez-Hernandez, H., M. W. Johnson and N. J. Reimer. 1999a. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). Biological Control 15: 145-152.

- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999b. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- Goolsby, J. A., M. Rose, R. K. Morrison and J. B. Woolley. 2000. Augmentative biological control of longtailed mealybug by *Chrysoperla rufilabris* (Burmeister) in the interior plantscape. *Southwestern Entomologist* 25: 15-19.
- Gray, R. A. 1952. Composition of honeydew extracted by pineapple mealybugs. *Science* 115: 129-133.
- Greathead, D. J. 1978. Biological control of Mealybug (Homoptera: Pseudococcidae) with special reference to cassava mealybug (*Phenacoccus manihoti* Mat-Ferr). pp. 70-80. *In* (K. F. Nwanje and K. Leuschner Ed.). Proceedings, International workshop on cassava mealybug *P. manihoti* Mat-Ferr (Pseudococcidae), June 26-29, 1977 Bas-Zaire, Zaire.
- Gross, H. R. 1994. Mass propagation of *Archytas marmoratus* (Diptera: Tachinidae). *Environmental Entomology* 23: 183-189.
- Gunasinghe, U. B. and T. L. German. 1986. Association of virus particles with mealybug wilt of pineapple. *Phytopathology* 76: 1073.
- Gunasinghe, U. B. and T. L. German. 1989. Purification and partial characterization of a virus from pineapple. *Phytopathology* 79: 1337-1341.
- HASS. 2002. Statistics of Hawaii Agriculture 2001. Hawaii Agricultural Statistics Services, Honolulu, HI.
- HDOA. 2001. List of active SLN registration. Pesticide Branch, Hawaii Department of Agriculture, Honolulu, HI.

- Herren, H. R. 1994. Cassava pest and disease management: An overview. *African Crop Science Journal* 2: 345-353.
- Higgins, J. E. 1912. Pineapple in Hawaii. *HAES Press Bulletin* 36: 30.
- Holldobler, B. and E. O. Wilson. 1990. *The Ants*. Harvard University Press, Cambridge, MA, 732 pp.
- Hu, J. S., D. M. Sether and D. E. Ullman. 1996. Detection of pineapple Closterovirus in pineapple plants and mealybugs using monoclonal antibodies. *Plant Pathology* 45: 829-836.
- Hu, J. S., D. M. Sether, X. P. Liu, M. Wang, F. Zee and D. E. Ullman. 1997. Use of a tissue blotting immunoassay to examine the distribution of pineapple closterovirus in Hawaii. *Plant Disease* 81: 1150-1154.
- Huang, T. and H. S. Chien. 1969. Chemical control of banana mealybug (*Dysmicoccus brevipes* (Cockerell)) (VII). *Plant Protection Bulletin Taiwan* 11: 123-132.
- Huebner, G. and W. Volkl. 1996. Behavioral strategies of aphid hyperparasitoids to escape aggression by honeydew-collecting ants. *Journal of Insect Behavior* 9: 143-157.
- Huffaker, C. B., R. L. Rabb and J. A. Logan. 1977. Some Aspects of Population Dynamics Relative to Augmentation of Natural Enemy Action, pp. 3-38. *In* *Biological Control by Augmentation of Natural Enemies* (R. L. Ridgeway and S. B. Vinson Ed.), Plenum Press, New York.
- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.

- Illingworth, J. F. 1931a. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Illingworth, J. F. 1931b. Longhorned grasshopper, *Conocephalus saltator* (Saussure), as a pest of pineapple in Hawaii. *Proceedings of the Hawaiian Entomological Society* 8: 407.
- Itioka, T. and T. Inoue. 1999. The alternation of mutualistic ant species affects the population growth of their trophobiont mealybug. *Ecography* 22: 169-177.
- Ito, K. 1938. Studies on the life history of the pineapple mealybug *Pseudococcus brevipes* (Ckll). *Journal of Economic Entomology* 31: 291-298.
- Jahn, G. C. and J. W. Beardsley. 1998. Presence absence sampling of mealybugs, ants, and major predators in pineapple. *Journal of Plant Protection in the Tropics* 11: 73-79.
- Jenkins, P. T. 1996. Free trade and exotic species introductions. *Conservation Biology* 10: 300-302.
- Johnson, M. O. 1935. *The Pineapple*. Paradise of the Pacific Press, Honolulu, 306 pp.
- Kairo, M. T. K., G. V. Pollard, D. D. Peterkin and V. F. Lopez. 2000. Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) in the Caribbean. *Integrated Pest Management Reviews* 5: 241-254.
- Kapadia, M. N., G. J. Parsana and P. G. Butani. 1995. Field recovery of *Anagyrus punctulatus* Agarwal, a parasitoid of the sugarcane mealybug. *Indian Sugar* 45: 361-362.



- Knipling, E. F. 1977. The theoretical basis for augmentation of natural enemy, pp. 79-123. *In* Biological Control by Augmentation of Natural Enemies (R. L. Ridgeway and S. B. Vinson Ed.), Plenum Press, New York.
- Kotinsky, J. 1910. Coccidae not hitherto recorded from these islands. Proceedings of the Hawaiian Entomological Society 2: 127-131.
- Larsen, L. D. 1910. Hawaiian Planters Record 3: 70-71.
- Lee, H. S. 1966. Ecological studies on pineapple mealybug (*Dysmicoccus brevipes* (Ckll.)) in relation to pineapple wilt. Plant Protection Bulletin of Taiwan 8: 211-219.
- Lee, H. S. and H. S. Chien. 1967. Seasonal abundance of mealybug (*Dysmicoccus brevipes* (Ckll.)) and its control on pineapple fruit. Plant Protection Bulletin of Taiwan 9: 59-66.
- Lim, W. H. 1972. Wilting and green spotting of pineapple by the bisexual races of *Dysmicoccus brevipes* Ckll. Malaysian Pineapple 2: 15-21.
- Lockhart, B. E. L., L. J. C. Autrey and J. C. Comstock. 1992. Partial purification and serology of sugarcane mild mosaic virus, a mealybug-transmitted closterolike virus. Phytopathology 82: 691-695.
- Luck, R. F., L. Nunney and R. Stouthamer. 1999. Sex ratio and quality in the culturing of parasitic Hymenoptera: a genetic and evolutionary perspective, pp. 653-672. *In* Handbook of Biological Control: Principles and Applications of Biological Control (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.
- Lynch, L. D., H. M. T. Hokkanen, D. Babendreier, F. Bigler, G. Burgio, Z. H. Gao, S. Kuske, A. Loomans, I. Menzler-Hokkanen, M. B. Thomas, G. Tommasini, J. K.

- Waage, J. C. V. Lenteren and Q. Q. Zeng. 2001. Insect biological control and non-target effects: a European perspective., pp. 99-125. *In Evaluating indirect ecological effects of biological control* (E. Wajnberg, J. K. Scott and P. C. Quimby Ed.), CABI Publishing, Wallingford, UK.
- McEwen, F. L., J. W. Beardsley, M. Hapai and T. H. Su. 1976. Laboratory tests with candidate insecticides for control of the big headed ant, *Pheidole megacephala* (Fabricius). *Proceedings of the Hawaiian Entomological Society* 23: 119-123.
- McKenzie, H. L. 1967. *Mealybugs of California*. University of California Press, Los Angeles, 525 pp.
- McNeely, J. A. 2000. The future of alien invasive species: Changing Social Views. *In Invasive Species in a Changing World* (H. A. Mooney and R. J. Hobbs Ed.), Island Press, Washington, D.C.
- Menezes, E., B. J. Suzuchi, L. B. Batista and A. J. Ismael. 1977. The use of granular insecticides for the control of the pineapple mealybug, *Dysmicoccus brevipes* (Cockerell, 1893) (Homoptera: Pseudococcidae). *Anais da Sociedade Entomologica do Brasil* 6: 287-294.
- Morrison, R. K. and E. G. King. 1977. Mass production of natural enemies, pp. 183-217. *In Biological Control by Augmentation of Natural Enemies: Insect and Mite Control with Parasites and Predators* (R. L. Ridgeway and S. B. Vinson Ed.), Plenum Press, New York.
- Neuenschwander, P. and W. N. O. Hammond. 1988. Natural enemy activity following the introduction of *Epidinocarsis lopezi* (Hymenoptera: Encyrtidae) against the

- cassava mealybug, *Phenacoccus manihoti* (Homoptera: Pseudococcidae), in southwestern Nigeria. *Environmental Entomology* 17: 894-902.
- Neuenschwander, P., C. Boavida, A. Bokonon-Ganta, A. Gado and H. R. Herren. 1994. Establishment and spread of *Gyranoidea tebygi* Noyes and *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae), two biological control agents released against the mango mealybug *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) in Africa. *Biocontrol Science and Technology* 4: 61-69.
- Neuenschwander, P. 1996. Evaluating the efficacy of biological control of three exotic homopteran pests in tropical africa. *Entomophaga* 41: 405-424.
- Neuenschwander, P. 2001. Biological control of the cassava mealybug in Africa: A review. *Biological Control* 21: 214-229.
- Nixon, G. E. J. 1951. *The Association of Ants with Aphids and Coccids*. CIE, London, 36 pp.
- Noyes, J. S. and M. Hayat. 1994. *Oriental Mealybug Parasitoids of the Anagyrini* (Hymenoptera: Encyrtidae). Natural History Museum/CAB International, London, 554 pp.
- Parella, M. P., L. S. Hansen and J. C. van Lenteren. 1999. Glasshouse environment, pp. 819-839. *In Handbook of Biological Control: Principles and Applications of Biological Control* (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic press, San Diego.
- Petty, G. J. 1987. Control of mealybugs and scale on pineapples by methyl bromide fumigation of planting material. *Phytophylactica* 19: 255-258.

- Py, C., J. J. Lacoëuilhe and C. Teisson. 1987. *The Pineapple Cultivation and Uses*. G. P. Maisonneuve et Larose, Paris, 568 pp.
- Rai, B. K. and A. K. Sinha. 1980. Pineapple: chemical control of mealybug and associated ants in Guyana. *Journal of Economic Entomology* 73: 41-45.
- Reimer, N., J. W. Beardsley and G. Jahn. 1990. Pest ants in the Hawaiian Islands, pp. 40-50. *In Applied Myrmecology. A World Perspective* (K. vander Meer, K. Jaffe and A. Cedeno Ed.), West View Press, Boulder, Colorado.
- Reimer, N. J. and J. W. Beardsley. 1990. Effectiveness of hydramethylnon and fenoxycarb for control of bigheaded ant (Hymenoptera: Formicidae), an ant associated with mealybug wilt of pineapple in Hawaii. *Journal of Economic Entomology* 83: 74-80.
- Reimer, N. J., M. Cope and G. Yasuda. 1993. Interference of *Pheidole megacephala* (Hymenoptera: Formicidae) with biological control of *Coccus viridis* (Homoptera: Pseudococcidae) in coffee. *Environmental Entomology* 22: 483-488.
- Reimer, N. J. 1994. Distribution and impact of alien ants in vulnerable Hawaiian ecosystems, pp. 11-22. *In Exotic Ants* (D. F. Williams Ed.), Westview Press, Boulder, Colorado.
- Reyd, G., B. L. Ru and B. Le Ru. 1992. Predation by *Hyperaspis raynevali* and *Exochomus flaviventris* larvae (Col. Coccinellidae) on cassava mealybug *Phenacoccus manihoti* (Hom. Pseudococcidae) colonies: Experimental study in laboratory. *Entomophaga* 37: 317-325.
- Rohrbach, K. G. and W. J. Apt. 1986. Nematode and disease problems of pineapple. *Plant Disease* 70: 81-87.

- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988. Mealybug wilt, mealybugs, and ants of pineapple. *Plant Disease* 72: 558-565.
- Sagarra, L. A. and D. D. Peterkin. 1999. Invasion of the Caribbean by the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). *Phytoprotection* 80: 103-113.
- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by the experiment station of the pineapple producers cooperative association during the period 1930-1938. *Proceedings of the Hawaiian Entomological Society* 10: 352.
- Sanches, N. F., R. C. Caldas and J. D. S. Souza. 1995. Efficiency of dimethoate in the control of the pineapple mealybug. *Anais-da-Sociedade-Entomologica-do-Brasil*. 24: 495-500.
- Santa Cecilia L. V, C., J. C. Matioli and A. I. Ciociola. 1992. Effects of climatic factors on the pineapple mealybug *Dysmicoccus brevipes* (Cockerell, 1893) (Homoptera, Pseudococcidae) in the principal production regions of the state of Minas Gerais. *Anais da Sociedade Entomologica do Brasil* 21: 135-145.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- Sether, D. M., A. V. Karasev, C. Okumura, C. Arakawa, F. Zee, M. M. Kislán, J. L. Busto and J. S. Hu. 2001. Differentiation, distribution, and elimination of two different Pineapple mealybug wilt-associated viruses found in pineapple. *Plant Disease* 85: 856-864.

- Sether, D. M. 2002. Pineapple Mealybug Wilt Associated Viruses: Vectors, Impacts, and Dynamics. Ph. D. Dissertation, University of Hawaii at Manoa, Honolulu.
- Sinclair, E. 1993. The pineapple plant. *In* Pineapple Pests and Disorders. (R. H. Broadley, R. C. Wassman and E. Sinclair Ed.) Department of Primary Industries. Queensland.
- Swezey, O. H. 1925. Records of introduction of beneficial insects into the Hawaiian islands. *Hawaiian planters records* 29: 368-378.
- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by the experiment station of the pineapple producers cooperative association during the period 1930-1938. *Proceedings of the Hawaiian Entomological Society* 10: 352.
- Thippaiah, M. and N. G. Kumar. 1999. *Dysmicoccus* sp. (Pseudococcidae: Homoptera): a pest of soybean in Karnataka. *Insect Environment* 5: 70.
- Ullman, D. E., T. L. German, C. E. McIntosh and D. D. F. Williams. 1991. Effect of heat treatment on a closteroviruslike particle associated with mealybug wilt of pineapple. *Fruits Paris* 46: 395-399.
- Ullman, D. E., D. F. Williams, H. Fleisch, J. S. Hu, D. Sether and A. Gonsalves. 1993. Heat treatment of pineapple: subsequent growth and occurrence of mealybug wilt of pineapple. pp. 407-410. *In* "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First international pineapple symposium, 2-6 November 1992 Honolulu, Hawaii.
- van Driesche, R. G. and T. S. Bellows. 1996. *Biological Control*. Chapman and Hall, New York, 539 pp.

- Waage, J. K., K. P. Carl, N. J. Mills and D. J. Greathead. 1985. Rearing entomophagous insects, pp. 45-66. *In Handbook of Insect Rearing Vol. I* (P. Singh and R. F. Moore Ed.), Elsevier, New York.
- Wagner, W. L., D. R. Herbst and S. H. Sohmer. 1999. *Manual of the Flowering Plants of Hawaii*. Bishop Museum, Honolulu, 1918 pp.
- Weber, P. W. 1955. Recent liberations of beneficial insects in Hawaii-IV. *Proceedings of the Hawaiian Entomological Society* 15: 635-638.
- Williams, D. J. and G. W. Watson. 1988. *The Scale Insects of Tropical South Pacific Region*. CAB International, Wallingford, 260 pp.
- Williams, D. J. and M. C. G. D. Willink. 1992. *Mealybugs of Central and South America*. CAB International, Wallingford, 635 pp.
- Williams, D. J. and H. Fleisch. 1993. Historical review of pineapple breeding in Hawaii. pp. 67-76. *In "Acta Horticulturae 334"* (D. P. Bartholomew and K. G. Rohrbach Ed.). *Proceedings, First international pineapple symposium, 2-6 November 1992 Honolulu, Hawaii*.
- Young, I. N. 1940. *The Hawaiian Islands and the Story of Pineapple*. American Can Company, New York, 48 pp.
- Zimmermann, C. E. 1948. *Insects of Hawaii*. University of Hawaii Press, Honolulu.

**III. ENHANCED PRODUCTION OF PINK PINEAPPLE MEALYBUG,  
*DYSMICOCCUS BREVIPES* (COCKERELL), (HOMOPTERA:  
PSEUDOCOCCIDAE)**

**INTRODUCTION**

Pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell), gray pineapple mealybug (GPM), *Dysmicoccus neobrevipes* Beardsley, and long tailed mealybug (LTM), (*Pseudococcus longispinus* (Targioni-Tozzetti)), (Homoptera: Pseudococcidae) are associated with pineapple, *Ananas comosus* (L) Merr., in Hawaii and other locations worldwide (Carter 1942; Collins 1960; German *et al.* 1992; Beardsley 1993; Sether *et al.* 1998). Compared to LTM, PPM and GPM are considered more important pests because of their ability to vector Pineapple Mealybug Wilt Associated Virus (PMWaV) (Sether *et al.* 1998). Mealybug wilt disease of pineapple (MWP) is the most widely distributed and most devastating disease of pineapple (Illingworth 1931; Rohrbach *et al.* 1988; Beardsley 1993; Ullman *et al.* 1993; Hughes and Samita 1998). Because MWP can only be expressed when both mealybugs (either species) and PMWaV are present, absence of either the mealybugs or virus will inhibit MWP expression (Hu *et al.* 1996; Hu *et al.* 1997; Hughes and Samita 1998). MWP control has principally been achieved by controlling the mealybugs either by insecticides or with natural enemies.

Introduction of coccinellid predators of pineapple mealybugs into Hawaii began in the early 1920s, but more rigorous introduction of natural enemies occurred during the 1930's. More than 20 predators and parasitoids of pineapple mealybugs were introduced into Hawaii (Sakimura 1939; Gonzalez-Hernandez *et al.* 1999). In ant-infested pineapple



plantings, natural enemies provide only partial control of mealybugs (Gonzalez-Hernandez *et al.* 1999). Several ant species tend PPM and GPM and feed on the honeydew they produce (Carter 1932; Gonzalez-Hernandez *et al.* 1999; Jahn and Beardsley 2000). The ants protect the mealybugs by actively guarding against the natural enemies as well as by building shelters around the mealybugs. (Illingworth 1931; Collins 1960; Jahn and Beardsley 1998) Mealybugs benefit from this association in two additional ways: a) the removal of honeydew which can accumulate and trap mealybugs (Gonzalez-Hernandez *et al.* 1999b); and b) from ant-assisted dispersal (Carter 1932; Rohrbach *et al.* 1988). Big-headed ant (BHA), *Pheidole megacephala* F. (Hymenoptera: Formicidae), is the most common attending ant in pineapple plantings in Hawaii below 600 m. The Argentine ant, *Linepithema humile* (Mayr), and imported fire ant, *Solenopsis geminata* (F.), are also reported occasionally in pineapple plantings (Illingworth 1931; Reimer and Beardsley 1990). Ant suppression results in high levels of mealybug mortality due to introduced natural enemies (Johnson 1935; Rai and Sinha 1980; Rohrbach *et al.* 1988; German *et al.* 1992; Gonzalez-Hernandez *et al.* 1999b).

After the withdrawal of organochlorine insecticides (e.g., mirex, chlordane and heptachlor), no formicide was registered for ant control in Hawaiian pineapple plantings (Reimer *et al.* 1990), except for Amdro® (Hydramethylnon) (American Cyanamid Co), which is permitted only on an annual emergency-use registration (Gonzalez-Hernandez 1995). The organophosphate diazinon is the only insecticide currently labeled for control of mealybugs on pineapple in Hawaii, and it has an uncertain fate due to the U.S. Food Quality Protection Act of 1996 (Rohrbach *et al.* 1988; FQPA 1996)

*Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae) is a highly specific, solitary endoparasitoid which attacks PPM. It was the most widely distributed natural enemy of PPM in Hawaii inflicting 0.3 – 9.9% mortality in commercial pineapple plantings where ants were present (Gonzalez-Hernandez *et al.* 1999). This parasitoid attacks all stages of PPM except the crawlers, but completes its life cycle only in the third instar and adult stages (Gonzalez-Hernandez 1995). Based on its abundance and host specificity as well as its persistence in attacking ant-attended mealybugs in the laboratory (H. Gonzalez-Hernandez, unpublished data), *A. ananatis* was selected as a potential candidate for an augmentative biological control program directed against PPM (Gonzalez-Hernandez 1995).

Augmentative biological control typically requires the ability to mass-produce the biological control agent (e.g., *Anagyrus ananatis*) as well as its host. Mass production of PPM was a prerequisite for the mass production of *A. ananatis* due to the parasitoid's high host specificity.

Pumpkins, taro, yams and potatoes are commonly used laboratory hosts for rearing mealybugs and scale insects (Haug *et al.* 1987; Gonzalez-Hernandez 1995; Maity *et al.* 1998; Sether *et al.* 1998). Citrus mealybug, *Planococcus citri* (Risso), and oleander scale, *Aspidiotus nerii* Bouche were mass produced on butternut pumpkins (Smith *et al.* 1996). Pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), and white tailed mealybug, *Ferrisia virgata* (Cockerell), were successfully mass reared on potato and potato sprouts respectively, in India (Gautam 1994). PPM has been reared for experimental purposes on butternut squash (Sether *et al.* 1998; Gonzalez-Hernandez *et al.* 1999), but the closely related GPM species was reared on Kobocho squash (Sether *et al.*

1998). Mass production techniques specific to PPM have not been developed, nor has ideal host plant material (e.g., squash fruit) been identified. The production of large numbers of PPM is not the only parameter to be considered in a rearing program intended to support parasitoid production. The availability of suitable PPM stages for parasitization as well as the production of suitably sized parasitoids is important. The size of the mealybug host has significant impact on *A. ananatis* adult size and sex ratio. Mature PPM adults are the most suitable host stage for maximum sized adult parasitoids with high fecundity (see Chapter 5).

Unlike many other mealybugs that lay eggs, *D. brevipes* does not lay eggs external to its body nor does it produce egg sacs. Crawlers emerge from the body of the female. In laboratory studies, Ito (1938) found that PPM (= *Pseudococcus brevipes* (Cockerell)) produced  $234 \pm 13$  crawlers in its life-time. In Hawaii, only female PPM individuals exist. This species also produces large quantities of honeydew rich in sugar and amino acids (Gray 1952; German *et al.* 1992). The honeydew is usually cleared away by attending ants in the field, but can accumulate on the mealybugs' plant host (e.g., squash) in the laboratory. Honeydew accumulation not only enhances mold growth, creating difficulties in handling plant hosts for production purposes, but also entraps PPM crawlers. Honeydew may be washed from the fruit, but this procedure can lead to high mealybug mortality as well as possible fungal problems due to increased moisture. Given that PPM typically lives below the soil surface on the roots of its plant hosts and that excreted honeydew is most likely absorbed by the surrounding soil, it was postulated that the submersion of PPM-infested squash in vermiculite might absorb

excess honeydew and significantly reduce levels of mealybug mortality from entrapment in honeydew.

The overall objective of this study was to improve methodologies for rearing PPM to provide suitable hosts for rearing *A. ananatis*. Specific goals were to: a) quantify amounts of honeydew produced by PPM reared on squash; b) evaluate the suitability of vermiculite to remove PPM-produced honeydew from host squash; c) evaluate the suitability of butternut and Kobocho squash for mass rearing PPM; d) evaluate the suitability of plastic and paper containers as rearing units for PPM colonies; and e) identify the optimum PPM stage(s) for initial squash infestation to produce uniformly large and mature adult PPM for parasitoid rearing.

## MATERIALS AND METHODS

PPM individuals used in these studies were obtained from a colony previously established on Kobocho squash, *Cucurbita maxima* Duch. Ex Lam. All studies were conducted at the Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa. Mature Kobocho squash fruit were used as the plant host unless otherwise stated. Squash were obtained from local grocery stores in Honolulu and washed with a 10% solution of commercial bleach, Clorox® (~0.525% sodium hypochlorite; Clorox Co. Ltd, Oakland, CA), for 10 minutes followed by rinsing and air-drying. Areas around the fruit pedicel and the terminal end were coated with melted household wax (Parowax™, Service assets Corporation, New Port Beach, CA) to reduce the incidence of mite infestations. All experiments were conducted at  $23 \pm 1^\circ\text{C}$ , 50-55% RH, and complete darkness.

### **Estimation of Honeydew Production**

Squash holding cages were made by rolling galvanized hardwire cloth into 12.5 cm long x 15 cm diameter cylinders. A 12 cm diameter plastic lid with an 8 cm diameter hole in the center was fitted to the cylinder about 2 cm above its base. One hundred third-instar and young adult PPM were manually transferred with a brush onto the terminal end of a butternut squash, *Cucurbita moschata* (Duch. Ex Lam) Duch ex Poir, and allowed to settle. The PPM infested squash was placed on the plastic lid so that the terminal end with mealybug-infested surface was exposed ventrally (Fig. 3.1). The squash was held in a temperature cabinet at  $23 \pm 1^\circ\text{C}$ ,  $50 \pm 5\%$  RH, and complete darkness. After two weeks, the number of settled mealybugs was reduced to 20 adult mealybugs per squash by removing excess mealybugs. A clean piece of aluminum foil (12 cm X 12 cm) was weighed and then placed under the infested squash within a 3.8 L ice cream carton (17 cm diameter x 18 cm; Neptune Paper Products Inc., Newark, NJ). After 24 hours, the aluminum foil was removed and the foil was reweighed to estimate the amount of honeydew present (i.e., amount of honeydew = weight of contaminated foil minus weight of clean foil). A clean piece of aluminum foil was placed back under the squash, and the process was repeated after 24 hours. Daily weights were taken over a 14 day period. The procedure was replicated with four squash to provide daily estimates for a total of 56 days. Mean daily weights of honeydew produced by individual adult PPM were estimated by calculating the arithmetic mean.

In a second study, a Kobocho squash was infested with 250 mg of adult mealybugs (ca. 300-400 individuals) and placed in a galvanized hardwire cloth squash holder. A 150 mm diameter petri dish lid was weighed and then placed within a 3.8 L ice

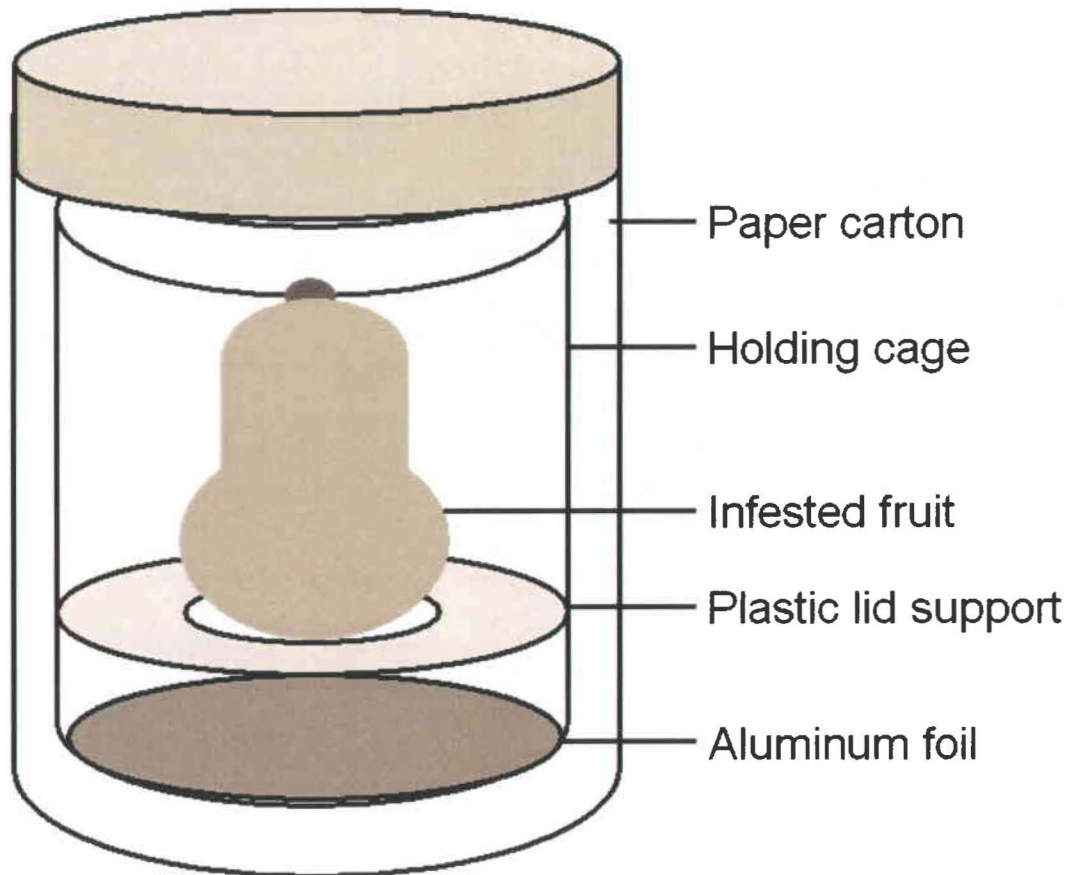


Fig. 3. 1. Squash setup for studying honeydew production by PPM. Twenty adult PPM were allowed to feed on ventral squash surface.

cream carton. The hardware cloth holder (as described above but without the plastic lid) with the infested squash was placed over the top of the petri dish lid. After one week, the petri dish lid was removed and weighed to allow estimations of accumulated honeydew, dead mealybugs, mealybug-produced wax and mealybug exuviae following the protocol in the previous experiment. The contaminated petri dish was replaced with a clean lid. This was done weekly for 10 weeks. The procedure was replicated four times. The mean weight of honeydew and other mealybug byproducts produced weekly was calculated.

### **Suitability of Vermiculite and Two Squash Varieties for PPM Production**

The suitability of two squash varieties, Butternut squash and Kobocho squash, was evaluated with and without vermiculite as an agent to remove excess PPM honeydew from fruit surfaces. The study was conducted in a 2 x 2 factorial design with five replications for each treatment. Squash were prepared as described above. All squash were individually placed in a hardware cloth holder with the pedicel end facing ventrally (Fig 3.2). The wire squash holder facilitated the frequent handling of the fruit for observation and data recording. The squash and hardware cloth holder were then placed in a 3.8 L paper ice-cream carton. Squash selected to be in the non-vermiculite treatment were not covered with vermiculite. Those selected for use in the vermiculite treatment were covered with coarse vermiculite (> 2.36 mm diameter) (Therm-O-Rock West Inc, AZ) except for ca. 10 cm of the upper most surface which was left uncovered. Squash with and without vermiculite had 250 mg of adult mealybugs (> 0.6 mm; ca. 300-400 individuals) gently sprinkled onto the bare surface of the fruit. For those squash receiving vermiculite, the mealybugs were later covered with a 2 cm layer of vermiculite, which totally covered the squash and PPM. Each ice cream carton was covered with a lid

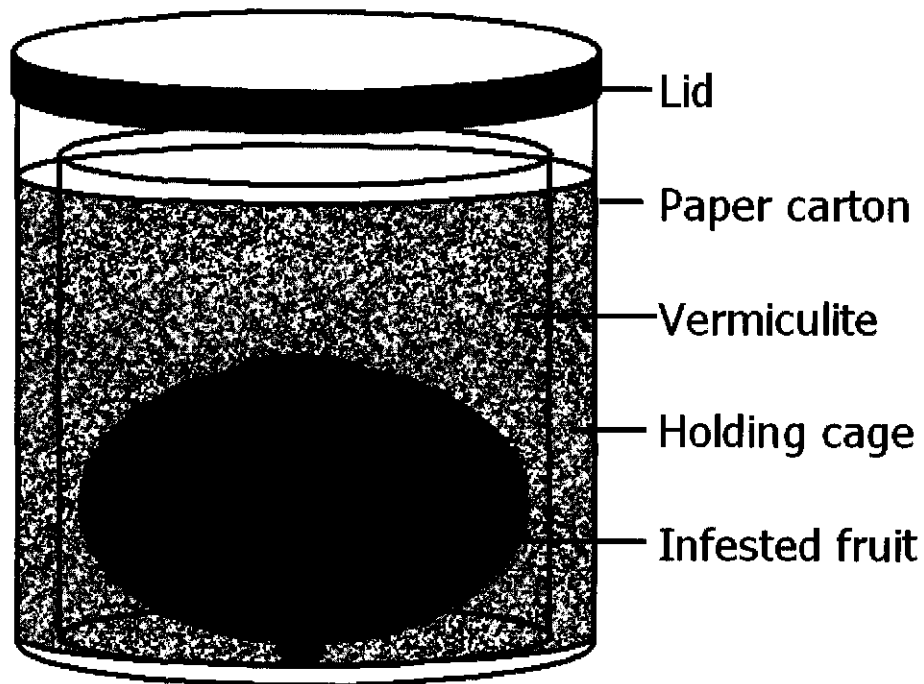


Fig. 3. 2. Experimental setup for the mass production of PPM. Kobocho squash with pedicel end down in a holding cage was placed in a 3.8 L paper carton. Squash was covered with coarse vermiculite (> 2.36 mm diameter) after infestation with mealybugs.



and held in a temperature cabinet. Each squash served as an experimental replicate. All squash were removed from the ice cream cartons once weekly for observation and data recording. Data collected on each squash included weight of squash, visual estimation of squash surface area covered by mealybugs each week, presence / absence of accumulated honeydew on squash surface, and appearance of the squash. For those squash submerged in vermiculite, vermiculite particles were brushed off the squash and combined with the vermiculite remaining in the carton. Vermiculite particles were mixed to uniformly disperse honeydew-saturated particles among the cleaner particles. Infested squash were then replaced in the containers. Those in the vermiculite treatment were covered with the contaminated vermiculite. All containers were replaced in the temperature cabinets until the following week or termination of the study. Mealybugs were harvested from a given squash when the first generation offspring of the original cohort of infesting mealybugs reached adulthood. Each squash was removed from the experiment upon mealybug harvest.

To facilitate removal or harvest of PPM individuals from infested fruit at the end of the experiment, the fruit were placed upon a hardware cloth screen within an open plastic shoebox (Rubbermaid, USA). The shoebox was placed under a fluorescent light (Phillips, 20 W) maintained about 25 cm above the fruit. Due to the presence of light, after about one hour most of the mealybugs dropped into the shoebox. The remaining mealybugs were gently brushed off the squash with a flat fan brush (No 6). PPM individuals were collected in the shoebox and poured through a stack of sieves (USA Standard Sieves No. 10, 20, 25, 30, 35, 40, and 60 (Fisher Scientific Inc., USA) with mesh openings equal to diameters of 2.0, 0.85, 0.71, 0.6, 0.5, 0.42, and 0.25 mm,

respectively) to separate out the various stages. Sieves were agitated to facilitate mealybug passage. The mass of mealybugs retained in each of the sieves was weighed.

*Statistical analysis.* One of the butternut squash in the vermiculite treatment became heavily infested with mites and rotted. Thus, it was discarded. The estimated percentage of squash fruit surface area covered by PPM individuals was square root transformed and subjected to general linear model (Proc GLM) (SAS Institute 1999) analysis to determine the effects of squash variety, vermiculite treatment, and time (week) after infestation (TAI) on mealybug production and their potential interactions. The weight of mealybugs produced per squash was standardized to the weight of mealybugs produced per kg of squash. Time to mealybug maturity and weight of total mealybugs and weight of mealybugs suitable for parasitoid production per kg squash were subjected to two-way Analysis of Variance (ANOVA) with squash variety and vermiculite treatment as independent variables (SAS Institute 1999).

### **Suitability of Plastic and Paper Containers as Rearing Units**

This experiment was conducted to compare the feasibility of using 5.7 L plastic containers (20 cm diameter X 19 cm; Hi-Plas Industries, CA) vs. 3.8 L paper ice cream cartons (17 cm diameter X 18 cm; Neptune Paper Products Inc., Newark, NJ) to rear PPM. Compared to paper cartons, plastic containers would last longer and be easier to clean than paper containers. However, closed plastic containers do not allow moisture to escape. Thus, plastic containers were not covered, in contrast to paper cartons. Squash were individually placed in hardware cloth fruit holders and misted with water to enhance mealybug establishment on fruit. Each squash was infested with 500 mg of immature mealybugs (0.25 mm > X < 0.5 mm: mostly crawlers, second instars, third instars, and

few young adults). The fruit holder with the infested squash was placed in either paper or plastic containers. Squash were then covered with coarse vermiculite (> 2.36 mm diameter) and held in a temperature cabinet as described above. Infested squash were handled and checked on a weekly basis as described above. The experiment was terminated when most of the original cohort reached adulthood and some of them began to produce offspring.

*Statistical analysis.* Percent surface area covered per squash was square root transformed and analyzed using a general linear model (Proc GLM) (SAS Institute 1999) with container type, TAI, and their potential interaction as independent variables. Time to mealybug maturity and weight of total mealybugs and weight of mealybugs suitable for parasitoid production per kg squash were subjected to one-way Analysis of Variance (ANOVA). The number of squash infested with the mite, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) was compared with Cochran-Mantel-Haenszel Chi-Square test (SAS Institute 1999).

### **Optimum PPM Stage for Initial Squash Infestation**

Because parasitoid size is affected by host mealybug size, and mature adult mealybugs produce large parasitoids with high fecundity and female-biased sex ratios (see Chapter 5), this study aimed to identify the optimum mealybug stage for squash infestation to produce uniformly large, mature adult mealybugs for *A. ananatis* propagation. Mealybugs were reared and harvested as described above and sized using sieves. Mealybug infestation stages were divided into three categories: immature mealybugs, young adults and mature adults. “Immatures” were considered those individuals  $0.25 \text{ mm} > X < 0.42 \text{ mm}$  (i.e., pass through sieve No. 35, but not Sieve No.

60) and were mostly third and second instars with a few crawlers and adults. “Young” adults were considered those individuals  $0.42 \text{ mm} > X < 0.6 \text{ mm}$  (i.e., pass through sieve No. 25, but not Sieve No. 35) which were not reproductively active; and “Mature” adults were  $0.6 \text{ mm} > X < 2.0 \text{ mm}$  (i.e., pass through sieve No. 10, but not Sieve No. 25) and reproductively active.

About 500 mg of mealybugs from each age category were used to infest individual squash. Those squash infested with immature mealybugs were lightly misted with water to enhance mealybug establishment. The squash were then placed in a hardware cloth holder and transferred to an ice cream carton and covered with coarse-grained vermiculite ( $> 2.36 \text{ mm}$ ). Establishment of adult stage mealybugs was not enhanced with a water mist, so fruit infested with adults were not misted. Infested squash were held in a temperature cabinet throughout the experiment and handled as described above.

The infested fruit were observed weekly. Two generations of mealybugs were distinguished. The mealybugs from the original infestation were categorized as Cohort 1 and their offspring as Cohort 2. The amount of squash surface area covered by the mealybugs was visually estimated. Any mites or molds observed were also recorded. The squash infested with mature mealybugs were harvested when most of the Cohort 2 reached mature adult and began to produce Cohort 3. Squash infested with young adults were harvested when most of Cohort 2 reached the young adult stage. Squash infested with immatures were harvested when the mealybugs began to reproduce Cohort 2. At the end of the experiment, mealybugs were harvested from each squash and passed through a stack of sieves as described earlier. The mealybugs retained in each of the sieves were

collected and weighed. Aliquots of individuals were taken from each mealybug stage category and the numbers of individuals in each stage (crawlers to adult) were counted to estimate the number of mealybugs per gram. From the estimated number of mealybugs per gram, mean weights of individual mealybugs in each category were estimated. Because parasitoids produced from mealybugs weighing < 0.6 mg had reduced fitness (e.g., reduced fecundity; unpublished data), only mealybugs larger than 0.6 mg were considered suitable for parasitoid production. Time to mealybug maturity was calculated by counting the days between the fruit infestation and mealybug harvest.

*Statistical analysis.* Percent surface area covered per squash was square root transformed and analyzed using a general linear model (Proc GLM) (SAS Institute 1999) with source mealybug type, TAI and their potential interaction as independent variables. Time to mealybug maturity and weight of total mealybugs were subjected to one-way Analysis of Variance (ANOVA). Weight of mealybug individuals retained by four sieve categories (pore opening diameter size 0.85, 0.71, 0.6 and 0.5 mm) was estimated and means compared by one way ANOVA within each sieve category. Weight of total mealybugs and weight of mealybugs suitable for parasitoid production per kg squash were subjected to one-way Analysis of Variance (ANOVA) (SAS Institute 1999).

## RESULTS

### **Quantification of Honeydew Production**

Mean daily honeydew production by an adult PPM over the experimental period (14 days) was  $0.41 \pm 0.018$  mg with a range from 0.35 to 0.50 mg (Fig. 3.3). There was no significant difference among the amounts of honeydew produced on different days.

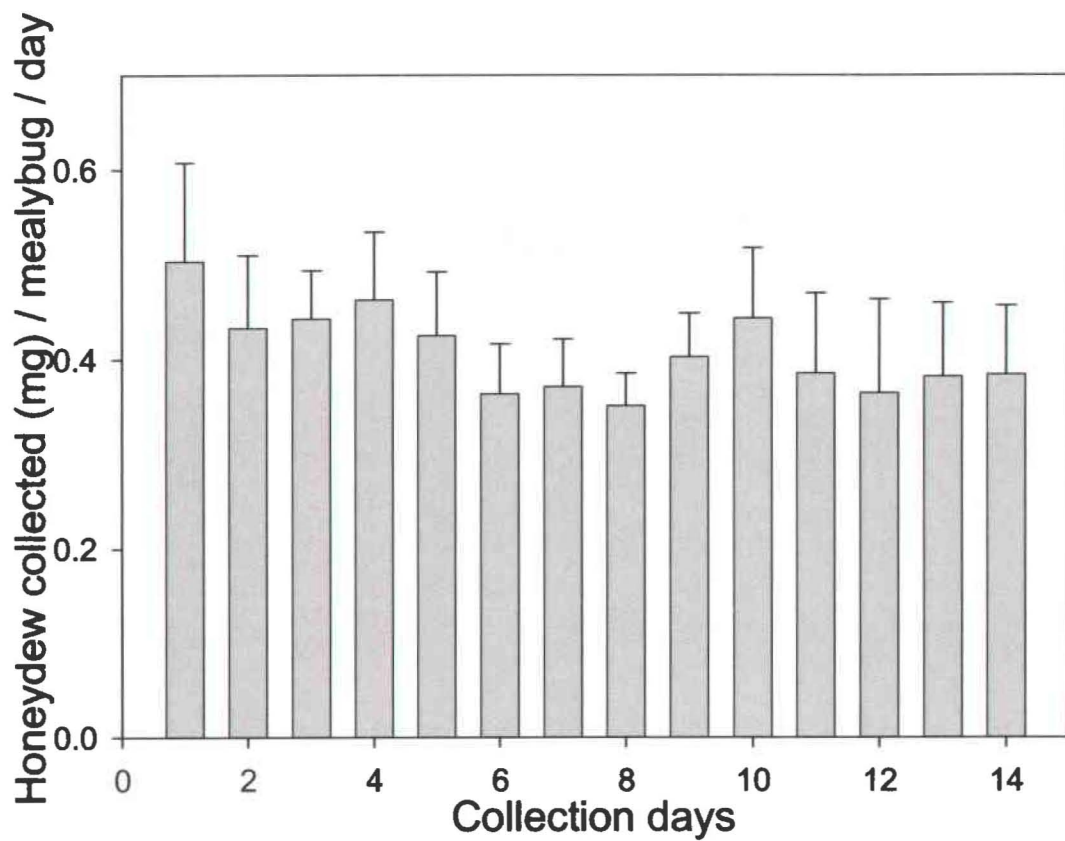


Fig. 3. 3. Mean weight of honeydew (Mean  $\pm$  SEM) produced by individual PPM adults per day.

The honeydew collected in this experiment contained large quantities of water, because very little time was allowed for evaporation before the honeydew was weighed.

In the second study, honeydew was observed on the surface of the squash within the first week, but none was collected from the petri dish. After the second week, honeydew, mealybug wax, mealybug exuviae, and mealybug bodies were collected from the petri dish. The honeydew collected consisted of a thick gummy, sticky material, produced after large amounts of water evaporated. As the number of mealybugs on the squash increased, much of the honeydew was ejected onto the container wall. The amount of honeydew collected per squash increased from 0.1 g in the second week to 1.4 g in the tenth week (Fig. 3.4).

#### **Suitability of Vermiculite and Two Squash Varieties for PPM Production**

There was a significant interaction between the squash variety and presence of vermiculite in terms of the squash surface area covered by the mealybugs at the initiation of the experiment as estimated by the intercept of the equations ( $F_{1,177} = 22.63, P < 0.0001$ ) (Fig 3.5). Butternut squash had a higher initial percent surface area covered than Kobocho squash when vermiculite was present, but it had a smaller surface area covered when the vermiculite was absent (Fig. 3.5). Despite the interaction, presence of vermiculite enhanced PPM settling on both squash varieties ( $F_{1,177} = 12.39, P = 0.0005$ ), but squash variety was not an important factor ( $F_{1,177} = 1.57, P = 0.2122$ ).

The rate of increase in the percent squash surface area covered by the mealybugs, as estimated by the slope of the equation (Fig 3.5), was significantly affected by the time after infestation ( $F_{1,177} = 1603.74, P < 0.0001$ ), but there was a significant interaction between the time after inoculation and the presence of vermiculite ( $F_{1,177} = 17.73, P <$

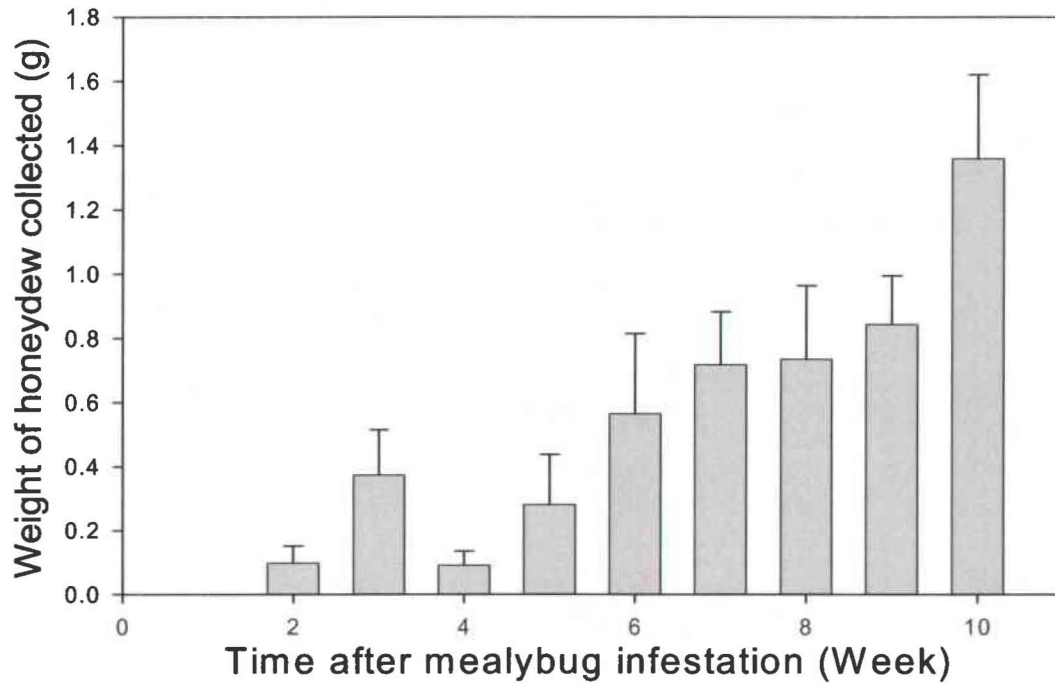


Fig. 3. 4. Mean weight ( $\pm$  SEM) of honeydew produced by PPM colony reared on a squash over 10 weeks.



0.0001) and time after inoculation and squash variety ( $F_{1,177} = 6.20, P = 0.0137$ ). The squash variety and presence of vermiculite significantly affected the rate of surface area coverage (Fig. 3.5)

In general, the presence of vermiculite promoted higher initial PPM establishment on the squash as well as a higher rate of increase per week compared to squash without vermiculite. The lowest initial establishment on the squash (Fig. 3.5, BC: Y-intercept = 0.1749) as well as the lowest rate of increasing surface coverage ( $b = 0.5738$ ) was recorded on the butternut squash without vermiculite (Fig. 3.5). In contrast, butternut squash with vermiculite had the highest initial establishment of mealybugs (Fig. 3.5, BV: Y intercept = 1.2715), but the highest increase rate was found with the Kobocho squash provided with vermiculite (Fig. 3.5, KV:  $b = 0.7886$ ). The results suggest that the use of vermiculite helped increase PPM establishment as well as increase the rate of increase of the mealybug population per individual squash as indicated by rate of surface coverage.

No significant differences were detected between the days to PPM maturity for mealybugs harvested from butternut and Kobocho squashes ( $F_{1,16} = 0.14, P = 0.7114$ ) (Table 3.1), but the use of vermiculite did significantly reduce the days to PPM maturity by 24.6 days (80.8 days without vermiculite vs. 56.2 days with vermiculite) ( $F_{1,16} = 112.65, P < 0.0001$ ). The total mean weight of PPM individuals produced per kg squash ranged from 4,099 mg (Butternut without vermiculite) to 6,201 mg (Kobocho without vermiculite) per kg squash, but there was no significant effect due to squash variety ( $F_{1,16} = 3.41, P = 0.838$ ) or the presence of vermiculite ( $F_{1,16} = 0.01, P = 0.9313$ ) (Table 3.1).

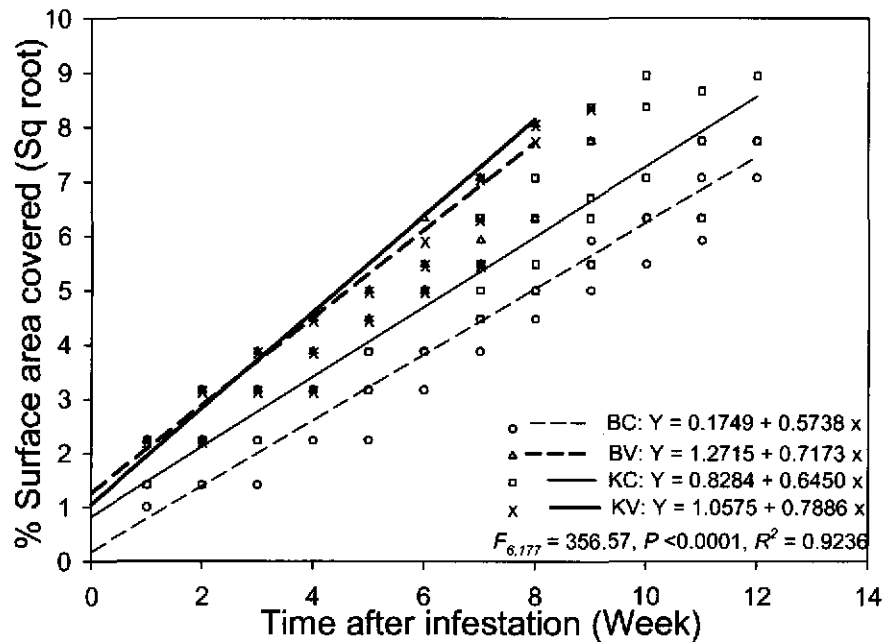


Fig. 3. 5. Establishment and increase in the surface area covered by PPM as affected by two squash varieties (Butternut and Kobocho) and treatment (presence and absence of vermiculite).

(BC: Butternut squash without vermiculite; BV: Butternut squash with vermiculite; KC: Kobocho squash without vermiculite; and KV: Kobocho squash with vermiculite).

[Squash variety:  $F = 1.57$ ,  $df = 1,177$ ,  $P = 0.2122$ ; Treatment:  $F = 12.39$ ,  $df = 1,177$ ,  $P = 0.0005$ ; TAI:  $F = 1603.74$ ,  $df = 1,177$ ,  $P < 0.0001$ ; Squash variety x Treatment:  $F = 22.63$ ,  $df = 1,177$ ,  $P < 0.0001$ ; Squash variety x TAI:  $F = 6.2$ ,  $df = 1,177$ ,  $P = 0.0137$ ; Treatment x TAI:  $F = 17.73$ ,  $df = 1,177$ ,  $P < 0.0001$ ).

Table 3. 1. Production of pink pineapple mealybug on butternut and Kobocho squash varieties with and without vermiculite provided in the rearing container to absorb honeydew.

Squash variety	Vermiculite	Mean No. ( $\pm$ SEM) days to MB maturity	Mean ( $\pm$ SEM) total weight (mg) of PPM individuals harvested per kg of squash	
			All individuals	Individuals > 0.6
Kobocho	Present	54.8 $\pm$ 3.2	5,662 $\pm$ 855	4,154 $\pm$ 655
	Absent	81.4 $\pm$ 2.4	6,201 $\pm$ 798	4,749 $\pm$ 797
Butternut	Present	62.6 $\pm$ 4.8	3,959 $\pm$ 670	2,588 $\pm$ 390
	Absent	80.2 $\pm$ 1.1	4,099 $\pm$ 872	3,746 $\pm$ 778

The mealybugs on squash without vermiculite were aggregated, mostly at the terminal end and a few cases also at the pedicel end, whereas those in vermiculite were dispersed around the terminal end. In the absence of vermiculite, mealybug-produced honeydew on the squash surface appeared as clear, crystalline droplets within the first week of infestation. By the second week, the honeydew coalesced and formed larger droplets, which began to drip from the squash surface and collect on the interior carton surface. Crawlers and young mealybugs as well as PPM-generated wax and exuviae frequently became entangled in the honeydew. Over time, the accumulation of honeydew, contaminated with wax, exuviae, and mealybug bodies, made the routine handling of the squash difficult. While harvesting PPM, few mealybugs would naturally fall from the squash. Brushing mealybugs off the squash was difficult because the brush would become sticky and possibly crush the mealybugs. The masses of mealybugs that were collected contained large proportions of dried remnants of honeydew, mealybug wax, and exuviae. In the presence of vermiculite, honeydew droplets did not accumulate. Mealybugs appeared clean and healthy throughout the study period, and while harvesting, most of them would readily fall from the squash when placed under the fluorescent light. Based on these results, Kobocho variety of squash with vermiculite was the treatment of choice.

#### **Suitability of Plastic and Paper Containers as Rearing Units**

Mealybugs established and fed on the squash in both kinds of containers. The overall model to explain the squash surface area covered by mealybugs with container type, time after infestation, and their interaction was highly significant ( $F_{3,75} = 92.2$ ,  $P < 0.0001$ ,  $R^2 = 0.7866$ ) (Fig. 3.6). The percentage of the surface area covered by PPM at

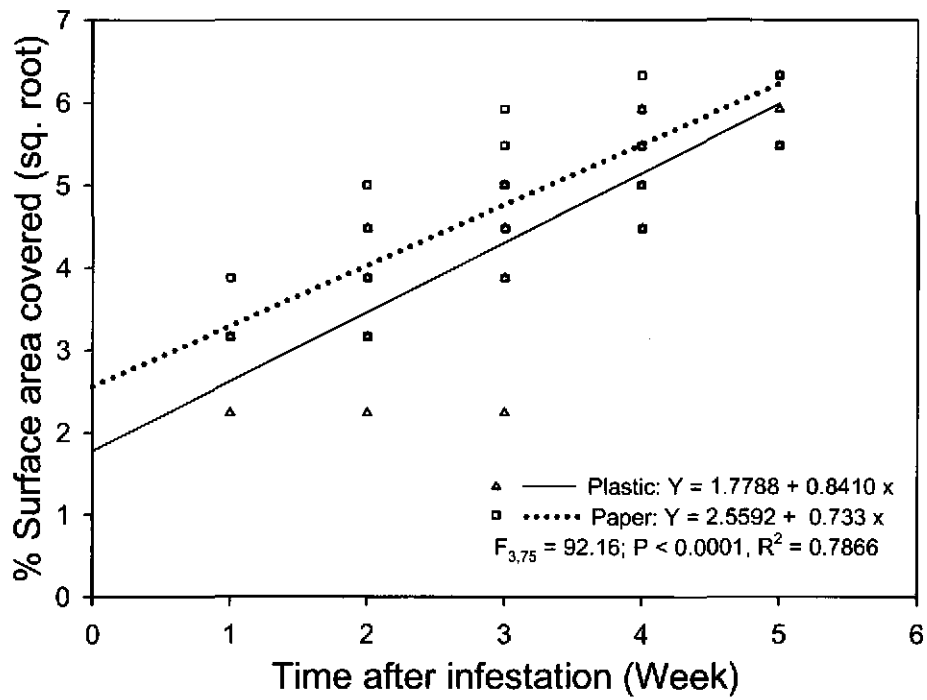


Fig. 3. 6. Effect of plastic and paper containers holding PPM infested squash relative to initial establishment and size of surface area covered by PPM.

(Container:  $F = 14.73, df = 1,75, P = 0.0003$ ; TAI:  $F = 260.52, df = 1,75, P < 0.0001$ ; Container x TAI:  $F = 1.23, df = 1,75, P = 0.2710$ ).

the initiation of the experiment (= Y intercept) was significantly higher for the paper container compared to the plastic container ( $F_{1,75} = 7.01$ ,  $P = 0.0099$ ) (Fig 3.6). Thus, more mealybugs settled on the squash in the paper container than in the plastic container.

The increase in the surface area covered by PPM individuals (based on slope of line in Fig. 3.6) was greatly dependent upon the time after infestation ( $F_{1,75} = 260.76$ ,  $P < 0.0001$ ), but not on the container type ( $F_{1,75} = 1.23$ ,  $P = 0.2710$ ). The mean time required by PPM to reach the mature adult stage in the plastic container ( $30.7 \pm 1.8$  days) was not significantly different from the paper container ( $29.6 \pm 1.1$  days) ( $F_{1,16} = 0.27$ ,  $P = 0.6088$ ) (Table 3.2).

Total mean quantity of mealybugs produced per unit weight of squash was significantly higher in the paper container ( $4,952 \pm 751$  mg) than in the plastic container ( $2,760 \pm 559$  mg) ( $F_{1,15} = 5.65$ ,  $P = 0.0312$ ) (Table 3.2). Similarly, the mean quantity of mealybug individuals appropriate for *A. ananatis* propagation ( $> 0.6$  mg) produced in the paper containers ( $4,339 \pm 685$  mg) was 83% higher than the mean quantity produced in plastic containers ( $2,370 \pm 535$  mg) ( $F_{1,15} = 5.24$ ,  $P = 0.0369$ ).

Infestation of host squash and feeding PPM by the mite *Tyrophagus putrescentiae* was the main problem associated with mass rearing PPM, and this was significantly increased in the plastic container (Cochran-Mantel-Haenszel Chi-square test:  $\chi^2 = 7.65$ ,  $df = 1$ ,  $P = 0.0057$ ). Eight of the nine squash held in the plastic container developed mite infestations whereas only one squash from the paper container was infested with mites. Based on these results, paper containers were superior to the plastic containers.

Table 3. 2. Effect of plastic and paper containers on the laboratory rearing of pink pineapple mealybug.

Rearing container	Mean No. ( $\pm$ SEM) days to MB maturity	Mean ( $\pm$ SEM) total weight (mg) of PPM individuals harvested per kg of squash	
		All individuals	Individuals > 0.6
Paper	29.5 $\pm$ 1.1 a	4,952 $\pm$ 751 a	4,339 $\pm$ 685 a
Plastic	30.7 $\pm$ 1.8 a	2,760 $\pm$ 559 b	2,370 $\pm$ 535 b

Values followed by the same letter within a column are not significantly different (P = 0.05).

### **Optimum PPM Stage for Initial Squash Infestation**

The increase in mealybugs per surface area of squash was significantly affected by the mealybug stage used for infestation and time after the inoculation. The initial percent surface area covered (= Y intercept) was significantly higher when immature ( $2.2295 \pm 0.1909$ ) and young adult ( $2.0299 \pm 0.1324$ ) PPM were used compared to mature adults ( $1.0803 \pm 0.1326$ ) (Fig. 3.7) ( $F_{2,350} = 17.86, P < 0.0001$ ). The rate of surface area covered by PPM was significantly affected by the TAI ( $F_{1,350} = 823.17, P < 0.0001$ ), however there was a significant interaction between TAI and the stage of mealybug used for infestation ( $F_{1,350} = 18.86, P < 0.0001$ ). The rate of increase of the surface area covered in immature mealybug treatment ( $b = 0.7538 \pm 0.0678$  per week) did not significantly differ from those in the young or mature adult infestations, but the rate was significantly higher for the mature adults ( $b = 0.8861 \pm 0.0291$  per week) compared to the young adult mealybug infestation ( $b = 0.6338 \pm 0.0290$  per week). Squash infested with immature mealybugs were ready for harvest within  $27.8 \pm 1$  days, which was significantly shorter than the time required when young adults ( $54.1 \pm 1.5$  days) and mature adults ( $53.8 \pm 1$  days) were used for infestation ( $F_{2,57} = 167, P < 0.0001$ ).

The sieve with a pore diameter of 2.0 mm did not retain any mealybug individuals, but removed most of the vermiculite particles. The largest mealybugs were retained by the sieve with pore size 0.85 mm, and the smaller mealybugs passed on to sieves with pore sizes 0.71, 0.6, 0.5, 0.42 and 0.25 mm. Many crawlers and mites passed through the sieve with an opening of 0.25 mm. The initial source of infestation affected the mean weight of the harvested mealybugs. The mean weight of mealybugs retained by sieve pore size 0.85 mm ranged from 1.69 mg to 2.11 mg. Mealybugs produced by initial



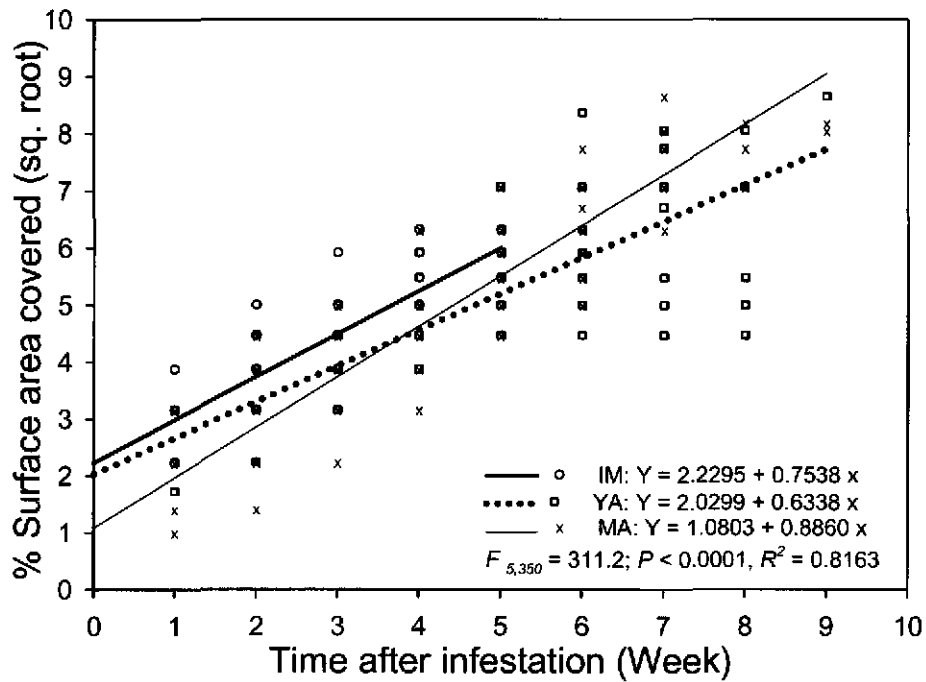


Fig. 3. 7. Effect of initial PPM inoculum stage.

(IM = Immatures; YA = Young adults; and MA = Mature adults) on the establishment and size of the surface area covered by PPM. (Inoculum stage:  $F = 17.86, df = 2, 350, P < 0.0001$ ; TAI:  $F = 823.17, df = 1, 350, P < 0.0001$ ; Inoculum stage x TAI:  $F = 18.86, df = 1, 350, P = 0.0001$ ).

infestation with immatures weighed significantly more than those produced from mature adults (2.11 mg vs. 1.69 mg, respectively) ( $F_{2,57} = 4.89$ ,  $P = 0.0109$ ) (Fig. 3.8). The mean weight of offspring produced from young adults (1.8 mg) was not significantly different from the other two treatments. The mealybugs retained by sieve pore size 0.71 mm ranged from 0.71 mg to 0.86 mg. In this category, offspring produced from mature adults (0.74 mg) were not different in weight from those produced from immatures or young adults. Mealybugs produced from immatures (0.86 mg) were significantly larger than the mealybugs produced by the young adults (0.71 mg) ( $F_{2,57} = 4.82$ ,  $P = 0.0116$ ).

Individuals retained by sieve No. 30 ranged from 0.35 to 0.56 mg, but no differences were detected among the three infestation sources ( $F_{2,57} = 2.58$ ,  $P = 0.0848$ ). Mean weight of the mealybugs retained by sieve No. 35 ranged from 0.22 to 0.54 mg.

Individuals produced by infesting squash with immatures were larger (0.54 mg) than those produced by infesting with young (0.22 mg) or mature adults (0.28 mg) ( $F_{2,57} = 6.8$ ,  $P = 0.0022$ ) (Fig 3.8). Infestation of the squash with immature stage mealybugs resulted in the harvest of relatively larger mealybugs than the other two infestation categories.

The use of sieves effectively separated the various mealybug stages. Sieve pore sizes of 0.85 and 0.71 mm effectively retained only mature adults in all treatments. Sieves with pore sizes of 0.6 and 0.5 mm retained high proportions of young adults when mature adults or immature mealybugs were used for initial infestation, but the proportion of immatures was highest when young adults (Fig. 3.9) were used for infestation. Mealybug individuals retained by the sieve with pore size 0.42 mm were proportionately similar in number of adult, second and third instars when mature adults or immature mealybugs were used for infestation. However, there were fewer adults than second and

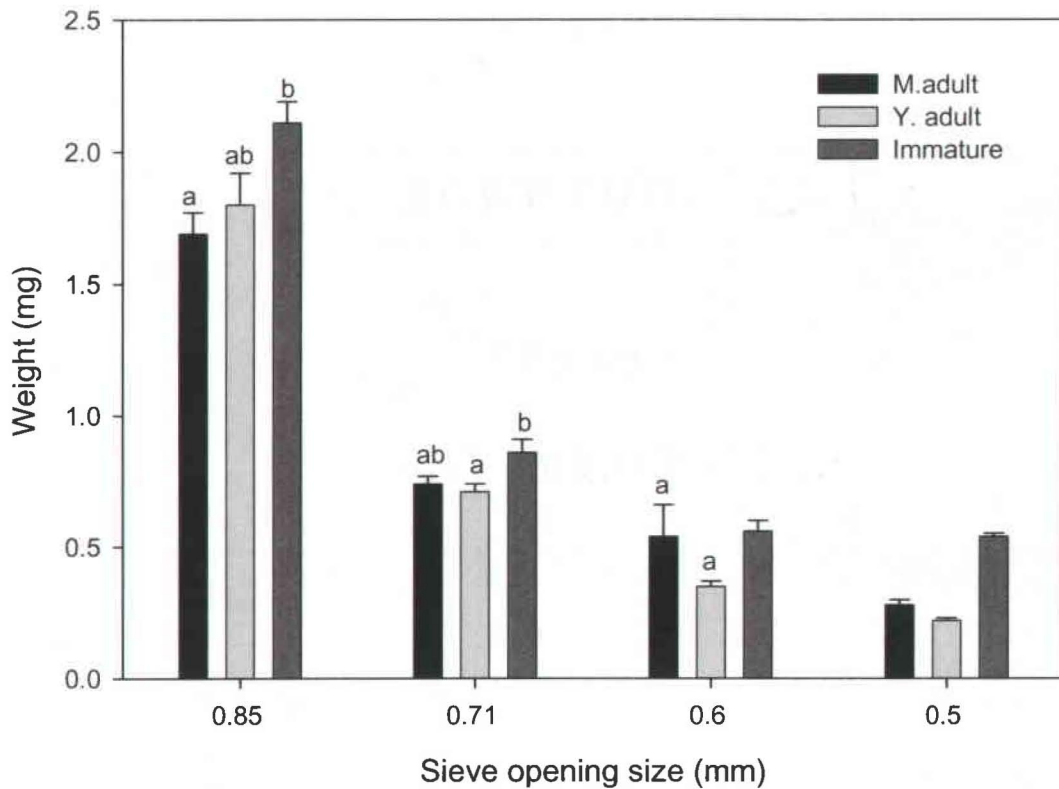


Fig. 3. 8. Mean weight (mg) of PPM individuals as affected by initial inoculum source.

(M. adult = Mature adult, Y. adult = Young adult, Immature = Immature stages of mealybugs) and grading through standard sieves. Columns with same letters within each sieve category are not significantly different from each other at P = 0.05.)

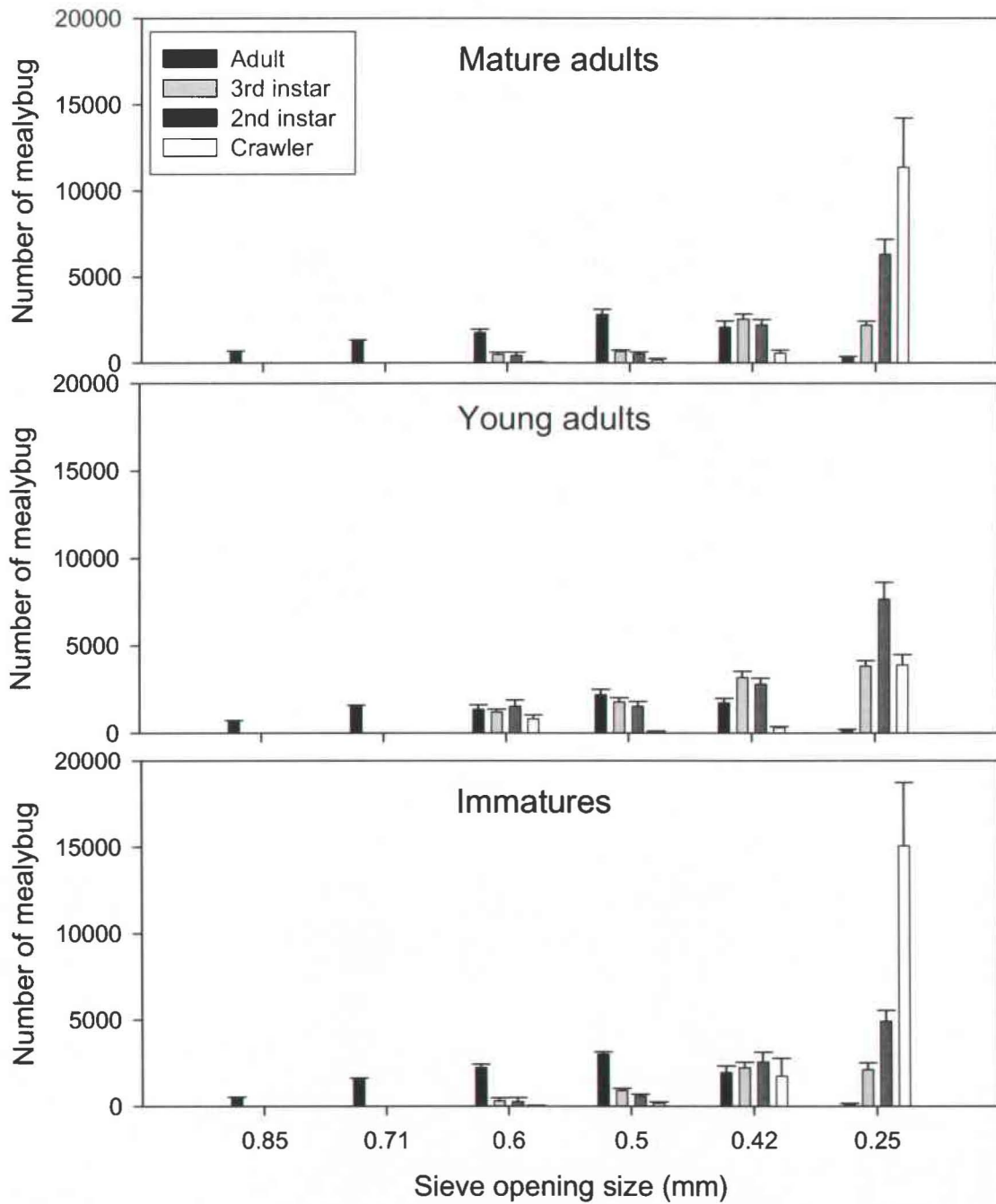


Fig. 3. 9. Proportionate distribution of different PPM stages produced by different infestation sources and separated by standard sieves (No 20 to 60).

third instars when young adults were used for infestation. The sieve with 0.25 mm pore diameter retained predominantly younger instars, whereas second instars prevailed among individuals produced from the young adult infestation compared to a prevalence of crawlers when immature and mature adults were used for infestation. Mean weight of mealybugs produced per kg squash was significantly less when young adults ( $2,887 \pm 398$  mg) were used for infestation as compared to mature adults ( $5,192 \pm 522$  mg) or immatures ( $4,142 \pm 428$  mg) ( $F_{2,57} = 6.5$ ,  $P = 0.0029$ ) (Table 3.3). The yield of mealybugs appropriate for *A. ananatis* propagation (individuals with weight > 0.6 mg) that were produced by infesting squash with mature adults ( $3,784 \pm 451$  mg) or immatures ( $3,644 \pm 393$  mg) were significantly greater than those produced from infestations of young adults ( $1,545 \pm 246$  mg) ( $F_{2,57} = 11.3$ ,  $P < 0.001$ ). The estimated number of mealybugs suitable for *Anagyrus* production (> 0.6 mg) produced per kg squash was significantly higher from squash infested with mature adults ( $2,257 \pm 255$ ) than squash infested with young adults ( $861 \pm 138$ ) or immatures ( $1,420 \pm 144$ ) ( $F_{2,57} = 14.1$ ,  $P < 0.0001$ ).

## DISCUSSION

PPM produced honeydew that initially accumulated on the squash surface and later dripped down into the container as a sticky, gummy liquid. Mealybug wax, exuviae and crawlers were found stuck on the honeydew when vermiculite was not used. Use of vermiculite removed the honeydew and weekly stirring of the contaminated particles dispersed the PPM-produced honeydew, wax, and molted casts, which gave the squash surface a clean and dry appearance in contrast to squash upon which the honeydew was

Table 3. 3. Effect of initial PPM infestation on PPM production in the laboratory.

Infestation stage	Mean No. ( $\pm$ SEM) days to MB maturity	Mean ( $\pm$ SEM) total weight (mg) of PPM individuals harvested per kg of squash		Mean No. ( $\pm$ SEM) Individuals $> 0.6$ mm mg
		All individuals	Individuals $> 0.6$ mg	
Immature	27.8 $\pm$ 1.0 b	4,142 $\pm$ 428 ab	3,644 $\pm$ 393 a	1,420 $\pm$ 144 b
Young adult	54.1 $\pm$ 1.5 a	2,887 $\pm$ 398 b	1,545 $\pm$ 246 b	861 $\pm$ 138 b
Mature adult	53.8 $\pm$ 1.0 a	5,192 $\pm$ 522 a	3,784 $\pm$ 451 a	2,257 $\pm$ 255 a

Values followed by same letter within a column are not significantly different from each other at P = 0.05.

permitted to accumulate. The mealybugs produced with vermiculite were easily harvested from the host squash, but mealybugs produced on squash without vermiculite were embedded within the honeydew making harvest difficult and inefficient. No obvious impediments to PPM reproduction were observed with the use of vermiculite. This is in contrast to the use of vermiculite to improve GPM production on the same squash varieties. GPM production decreased when squash were submerged in vermiculite (M. W. Johnson, personal observations). However, the microenvironment of the rearing chamber needs to be well ventilated and the containers need to be permeable to moisture. The paper containers used in the study were permeable to moisture whereas the plastic containers were not, leading to the accumulation of moisture in the plastic containers, thereby making the microenvironment within the plastic containers more congenial for mite infestation. In the plastic containers, mite infestation generally began on the squash surface closest to the bottom of the container. There was probably more moisture in the bottom of the plastic containers because the containers were uncovered. The greater amount of mealybugs produced in the paper containers may have been due to the higher initial settlement of mealybugs on squash.

Potatoes, melons and squash are commonly used for rearing many scales and mealybugs (Etzel and Legner 1999). [Papacek, 1985 #1490] found butternut squash (*C. moschata*) to be superior to other squash for rearing oleander scale. The citrus mealybug, *Planococcus citri* Risso, was successfully reared on *Cucurbita moschata* and up to 12,000 mealybugs could be produced per squash (Krishnamoorthy and Singh 1987; Smith *et al.* 1996).

On average, the PPM immature stage and pre-oviposition adult stage are 33 and 27 days, respectively (Ito 1938). When immature mealybugs were used for infestation, the mature mealybugs were ready for harvest in about four weeks (Table 3.3 and 3.4). Therefore, mature adults must have developed from third instar or young adult mealybugs (as crawlers and second instars would develop only to third instar or young adult stage in the four weeks of incubation). Inoculation of fresh squash with mature PPM adults resulted in the production of the highest number of individuals appropriate for parasitoid production. When mature adults were used for inoculation, they immediately began to reproduce progeny. The resulting progeny matured synchronously. The proportion of mealybugs that were not suitable for parasitoid production, due to their smaller size, were least (ca. 12%) in the immature treatment and greatest (ca. 48 %) in the young adults. Infestation with mature adults produced a relatively high proportion (72 %) of mealybugs suitable for parasitoid production (Table 3.3). The smaller mealybugs that are not suitable for parasitoid production have to go through a re-infestation procedure onto fresh squash, which will eventually produce mealybugs of suitable sizes for parasitoid production in two to four weeks (Table 3.3). The mature adult mealybugs produced by infesting a squash with young adult and immature stages of PPM were larger than the adults produced by infesting the squash with mature adults (Fig. 3.6) These larger mealybugs can be used to infest new squash (as mature adults) or used to propagate parasitoids which will be large and highly fecund (see Chapter 5 for more details).

The number of mealybugs produced for parasitoid propagation was highest (ca. 2,200 individuals) when mature adults were used for inoculation. This quantity was



smaller than the number (ca. 12,000 individuals) of *Planococcus citri* reared on butternut squash for mass production of parasitoid *Leptomastix dactylopii* (Smith *et al.* 1996). Third instar *P. citri* was the most appropriate stage to expose to the parasitoid *L. dactylopii*. This host stage required only 21 days on the squash. *A. ananatis* prefers to attack PPM adults (Gonzalez-Hernandez 1995) and requires mature adults to produce highly fecund adults (R. R. Pandey, unpublished data). It requires about eight weeks for PPM to produce adult mealybugs on squash. Due to the time difference, fewer PPM can be produced per squash than *P. citri*. [Papacek, 1985 #1490] suggested that 1.5 - 2 tons of pumpkin would need to be infested per week to produce 15-20 million individuals of *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae) per year for control of California red scale, *Aonidiella aurantii* (Maskel) (Homoptera: Diaspididae). This would yield < 200 parasitoids per kg squash used. Results reported here are better than that reported for production of *A. lingnanensis*. Efforts should be placed on maximizing the rate of mealybug parasitization to maximize parasitoid production.

Kobocha squash is more appropriate for mass rearing purposes to produce more mature adult mealybugs suitable for parasitoid rearing per unit weight of the substrate. Use of vermiculite effectively removed honeydew, wax, and exuviae from PPM infested squash surfaces. Because squash surfaces were clean and dry, harvest of the mealybugs was easier in the vermiculite treatment. The plastic container tested should be avoided due to increased mite infestations compared to the paper containers. Infesting 600-700 g squash with about 300 (0.5 g) adult mealybugs would produce nearly 1500 mature adult mealybugs in 7 to 8 weeks at a temperature of  $23 \pm 1^{\circ}\text{C}$ . With an average market price of

Kobocho squash at \$ 3.00 per kg fruit, each dollar value of squash would produce more than 700 adult PPM suitable for *A. ananatis* production.

## REFERENCES

- Beardsley, J. W. 1993. The pineapple mealybug complex: taxonomy, distribution and host relationships. pp. 383-386. *In* "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First International Pineapple Symposium, 2-6 November, 1992 Honolulu, Hawaii.
- Carter, W. 1932. Pineapple mealybug, *Pseudococcus brevipes* (Ckll), occurring on pineapple plants. *Ecology* 13: 296-304.
- Carter, W. 1942. The geographical distribution of mealybug wilt with notes on some other insect pests of pineapple. *Journal of Economic Entomology* 35: 10-15.
- Collins, J. L. 1960. *The Pineapple: Botany, Cultivation and Utilization*. Leonard Hill, London, 295 pp.
- Etzel, L. K. and E. F. Legner. 1999. Culture and colonization, pp. 125-198. *In* *Handbook of Biological Control: Principles and Applications of Biological Control* (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.
- Food Quality Protection Act. 1996. Food quality protection Act of 1996. USC 136. 55 pp.
- Gautam, R. D. 1994. *Biological Pest Suppression*. Westvill Publishing House, New Delhi, 221 pp.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. *Advances in Disease Vector Research* 9: 241-259.
- Gonzalez-Hernandez, H. 1995. *The Status of Biological Control of Pineapple Mealybugs in Hawaii*. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.

- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- Gray, R. A. 1952. Composition of honeydew extracted by pineapple mealybugs. *Science* 115: 129-133.
- Haug, T., H. R. Herren, D. J. Nadel and J. B. Akinwumi. 1987. Technologies for the mass rearing of cassava mealybugs, cassava mites and their natural enemies. *Insect Science and its Application* 8: 879-881.
- Hu, J. S., D. M. Sether and D. E. Ullman. 1996. Detection of pineapple Closterovirus in pineapple plants and mealybugs using monoclonal antibodies. *Plant Pathology* 45: 829-836.
- Hu, J. S., D. M. Sether, X. P. Liu, M. Wang, F. Zee and D. E. Ullman. 1997. Use of a tissue blotting immunoassay to examine the distribution of pineapple closterovirus in Hawaii. *Plant Disease* 81: 1150-1154.
- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.
- Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Ito, K. 1938. Studies on the life history of the pineapple mealybug *Pseudococcus brevipes* (Ckll). *Journal of Economic Entomology* 31: 291-298.
- Jahn, G. C. and J. W. Beardsley. 1998. Presence absence sampling of mealybugs, ants, and major predators in pineapple. *Journal of Plant Protection in the Tropics* 11: 73-79.

- Jahn, G. C. and J. W. Beardsley. 2000. Interactions of ants (Hymenoptera: Formicidae) and mealybugs (Homoptera: Pseudococcidae) on pineapple. Proceedings of the Hawaiian Entomological Society 34: 181-185.
- Johnson, M. O. 1935. The Pineapple. Paradise of the Pacific Press, Honolulu, 306 pp.
- Krishnamoorthy, A. and S. P. Singh. 1987. Biological control of Citrus mealybug, *Planococcus citri*, with an introduced parasitoid, *Leptomastix dactylopii* in India. Entomophaga 32: 143-148.
- Maity, D. K., A. K. Sahoo and S. K. Mandal. 1998. Evaluation of laboratory hosts for rearing and mass multiplication of *Planococcus minor* (Maskell) (Pseudococcidae: Hemiptera). Environment and Ecology 16: 530-532.
- Rai, B. K. and A. K. Sinha. 1980. Pineapple: chemical control of mealybug and associated ants in Guyana. Journal of Economic Entomology 73: 41-45.
- Reimer, N., J. W. Beardsley and G. Jahn. 1990. Pest ants in the Hawaiian Islands, pp. 40-50. In Applied Myrmecology. A World Perspective (K. vander Meer, K. Jaffe and A. Cedeno Ed.), West View Press, Boulder, Colorado.
- Reimer, N. J. and J. W. Beardsley. 1990. Effectiveness of hydramethylnon and fenoxycarb for control of bigheaded ant (Hymenoptera: Formicidae), an ant associated with mealybug wilt of pineapple in Hawaii. Journal of Economic Entomology 83: 74-80.
- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988. Mealybug wilt, mealybugs, and ants of pineapple. Plant Disease 72: 558-565.
- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by the experiment station of the pineapple producers cooperative association during

- the period 1930-1938. Proceedings of the Hawaiian Entomological Society 10: 352.
- SAS Institute. 1999. The SAS System for Windows release 8.0. SAS Institute, Cary, NC, USA.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- Smith, D., C. G. Freebairn and D. F. Papacek. 1996. The effect of host density and parasitoid inoculum size on the mass production of *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) and *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae) in Queensland. *General and Applied Entomology* 27: 57-64.
- Ullman, D. E., D. F. Williams, H. Fleisch, J. S. Hu, D. Sether and A. Gonsalves. 1993. Heat treatment of pineapple: subsequent growth and occurrence of mealybug wilt of pineapple. pp. 407-410. *In* "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First international pineapple symposium, 2-6 November 1992 Honolulu, Hawaii.

#### IV. BIOLOGICAL PARAMETERS OF *ANAGYRUS ANANATIS* GAHAN (HYMENOPTERA: ENCYRTIDAE)

##### INTRODUCTION

Mealybug wilt disease of pineapple (MWP) is the most important disease of pineapple worldwide (Carter 1963; Gunashinghe and German 1986; Hu *et al.* 1996; Hu *et al.* 1997). Pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell), and gray pineapple mealybug (GPM), *Dysmicoccus neobrevipes* Beardsley (Homoptera: Pseudococcidae), are the primary vectors of Pineapple Mealybug Wilt Associated Virus (PMWaV) (Sether *et al.* 1998). PPM is the most commonly encountered mealybug in Hawaiian pineapple plantings and the most widely distributed mealybug worldwide (Williams and Watson 1988; Gonzalez-Hernandez *et al.* 1999b). The PMWaV in association with PPM and/or GPM feeding leads to the expression of typical MWP symptoms and plant death, if mealybugs are not controlled (Carter 1945, 1963; German *et al.* 1992; Hu *et al.* 1996; Hughes and Samita 1998). Introduction of biological control agents from South and Central America, where these mealybugs originated, provided partial control of the mealybugs in Hawaii when ants were present (Collins 1960; Beardsley 1993; Gonzalez-Hernandez *et al.* 1999b). *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae) was the most common natural enemy of PPM found in Hawaiian pineapple plantings (Gonzalez-Hernandez *et al.* 1999b). It was present in all pineapple fields surveyed in Hawaii by Gonzalez-Hernandez *et al.* (1999b) and parasitized ant-attended mealybugs.

Three ant species, big headed ant, *Pheidole megacephala* F., Argentine ant, *Linepithema humile* (Mayr) and fire ant, *Solenopsis geminata* (F.) (Hymenoptera: Formicidae), are commonly found in the pineapple agroecosystem in Hawaii and attend PPM and GPM for honeydew (Reimer *et al.* 1990; Reimer 1994). These ants, especially *P. megacephala*, have been blamed for dispersing mealybugs as well as protecting them against their natural enemies while removing the excess honeydew produced by the mealybugs (Illingworth 1931; Carter 1932; Jahn and Beardsley 1998; Gonzalez-Hernandez *et al.* 1999a). Elimination of attending ants from pineapple fields has led to subsequent improved suppression of the mealybugs by their natural enemies and prevented MWP development (Rai and Sinha 1980; Beardsley *et al.* 1982; Petty and Tutsin 1993).

After the cancellation of mirex and other organochlorine insecticides for use in pineapple, no effective formicides remained registered for use in pineapples in Hawaii (Reimer *et al.* 1990). Amdro® (hydramethylnon, American Cyanamid Co.) provided effective ant control (Reimer and Beardsley 1990), but approval for its application is annually granted on an 'emergency needs basis' (Section 18) (HDOA 2001). Alternative pest management techniques are necessary if approval for Amdro® is denied for commercial pineapple production. They are also needed for organic pineapple production where synthetic chemicals are not allowed. Developing integrated pest management techniques by maximizing biological control will help minimize dependencies on chemical pesticides.

*Anagyrus ananatis* is a solitary endoparasitoid of PPM, which successfully established in Hawaiian pineapple fields after its introduction in 1935-1937 from Brazil



(Funasaki *et al.* 1988; Gonzalez-Hernandez 1995). Although de Santis (1964, 1979, 1980) (as cited by Noyes and Hayat 1994) reported rhodes grass mealybug, *Antonina graminis* (Maskell), stripped mealybug, *Ferrisia virgata* (Cockerell), and citrus mealybug, *Planococcus citri* (Risso), as other hosts for *A. ananatis*, it has only been reared from PPM in Hawaii. In a laboratory study, *A. ananatis* also attacked GPM, but PPM was preferred almost 13-fold more than GPM in a choice test, but surprisingly failed to attack any GPM under no choice conditions (Gonzalez-Hernandez 1995). *Anagyrus ananatis* completes its life cycle in about 23 days at 26°C and produces a mean of 27.7 progeny over a mean lifespan of 9.8 days (Gonzalez-Hernandez 1995). Based on its prevalence, host specificity, and persistence in host attack, *A. ananatis* was identified as a potential candidate for augmentative biological control of PPM (Gonzalez-Hernandez 1995). Development of mass production techniques for *A. ananatis* to facilitate evaluation of augmentative biological control of PPM is underway at the University of Hawaii at Manoa. Little information was available on the biology and behavior of *A. ananatis* to support its large scale production.

The overall objective of the studies reported herein was to elucidate various aspects of *A. ananatis* biology that will be useful in the development of mass production techniques. The specific objectives of the studies reported herein were to: a) determine the effect of food supplements (pure honey and honey solutions) on the longevity of *A. ananatis*; b) evaluate the effect of starvation on adult longevity; and c) to determine the periodicity of emergence of *A. ananatis* adults from the mealybug mummies and the periodicity of oviposition by adult females.

## MATERIALS AND METHODS

PPM individuals from a colony of Hawaii's uniparental (female only) PPM population were obtained from a culture maintained at the Department of Plant and Environmental Protection Sciences (PEPS), University of Hawaii at Manoa. *Anagyrus ananatis* individuals used in these studies were initially collected from Kunia, Oahu, HI, in November 1998 and then laboratory reared using adult female PPM as hosts.

### **Factors Affecting Parasitoid Longevity**

All experiments were conducted in polystyrene tube cages (19 cm length X 8.3 cm diam.). Horizontally set tubes were fitted with fine mesh organza (36 mesh/cm) (Fabric Mart, Honolulu, HI) on one end for ventilation and covered with a polystyrene petri dish lid on the other end. Experiments were conducted within a temperature cabinet maintained at  $24 \pm 1^\circ\text{C}$ ,  $55 \pm 5\%$  RH, and 14:10 (L: D) hours photophase.

*Honey and water.* This study was conducted from August to October 1999 in a 2 x 2 factorial design. The first factor was presence or absence of honey and the second factor was presence or absence of water. The treatments were 1) water only; 2) honey only; 3) honey and water; and 4) neither water nor honey (control). Water was provided through a 38 x10 mm cotton dental wick (Henry Schein, Middlesex, England) inserted into 28 ml diet cup (Solo Cup Company, Urbana, IL). A honey droplet (SueBee Clover Honey, Sioux Honey Association, Sioux City, Iowa) was provided on a clean glass microscope slide.

Three female and two male *A. ananatis* adults were transferred to a polystyrene tube cage on the day of their emergence. Water and honey were replenished as needed in the respective treatments. Numbers of surviving individuals were recorded daily until all

died. The study was repeated ten times. Data were  $\text{Log}_{10}$  transformed to normalize the variance distribution and analyzed with general linear models (SAS Institute 1999) with sex, honey and water and their interactions as the independent variables.

*Concentration of honey solution.* This study was conducted from January to March 2001. Treatments were 1) honey only; 2) 50% honey water solution; 3) 25% honey water solution; and 4) 10% honey water solution; and 5) water only (control). Water and honey concentrations were provided on saturated dental cotton wicks.

Five female *A. ananatis* adults were placed in each polystyrene tube on the day of their emergence, and the numbers of surviving wasps in each tube were recorded daily until all died. The study was repeated three times. Data were  $\text{Log}_{10}$  transformed to normalize the distribution of the variance and analyzed using ANOVA procedures (SAS Institute 1999).

*Starvation.* This study was conducted from May to June 2001. Five newly emerged (between 0930 and 1000 Hours) female adults were collected and transferred to a polystyrene tube cage provisioned with water. Treatments were starvation for: 1) six hours; 2) 12 hours; 3) 24 hours; and 4) 48 hours after which time all individuals were provided with excess honey and water. A control was provided consisting of excess honey and water from the time individuals were placed into the experimental unit (i.e., tube). Treatments were repeated five times. The number of surviving wasps in each tube were recorded daily until all died. Data were  $\text{Log}_{10}$  transformed to normalize the distribution of the variance and analyzed using ANOVA procedures (SAS Institute 1999).

*Effect of prior feeding on progeny production.* This experiment was conducted to evaluate if provision of honey to newly emerged *A. ananatis* females, prior to their access

to mealybugs, would increase their post-feeding longevity and subsequent progeny production. Thirty-six individual pairs of laboratory-reared *A. ananatis* adults were transferred to separate polystyrene tube cages on the day of their emergence. Water was provided using a moistened cotton wick. For each of nine mated pairs, a honey droplet was provided on a clear glass slide for either one, two or four days from the day of emergence. Only after receiving their total allotment of honey (i.e., 1, 2 or 4 days) were the females provided PPM hosts, and no additional honey was provided. Every 24 hours, a new group of 25 PPM adults were provided to each female, and the previously exposed mealybugs were removed until all female parasitoids died. The longevity of females in all treatments was recorded. All potentially parasitized PPM adults were held in a temperature cabinet for two weeks after exposure, at which time all mummified PPM adults were counted. These treatments were compared to nine mated *A. ananatis* pairs not receiving any honey (i.e., untreated control). Twenty-five PPM adults were provided to the control group on the day of their emergence, and these hosts were replaced every 24 hours until the females died. All treatments were replicated nine times except for the four day honey treatment experiment which was replicated seven times because two females died before the completion of study.

The number of mummies produced by each *A. ananatis* female during its lifetime was log transformed and analyzed using ANOVA. The post-feeding longevity (after being deprived of honey) of the female parasitoids was also analyzed by ANOVA procedures (SAS Institute 1999).

## Periodicity of Biological Events

*Ovipositional behavior.* This study was conducted to determine the influence of photoperiod on ovipositional behavior, and, in particular, to determine if *A. ananatis* parasitizes its host during the scotophase. Laboratory colonies were normally held under 14 hours photophase and 10 hours scotophase, however, it was unknown what time of the day that *A. ananatis* normally parasitized PPM. To determine the optimum time period(s) for exposing mealybugs to *A. ananatis* to obtain maximum parasitization, five hourly periods were assessed. The photophase periods were determined by dividing the 14 hour photophase (0830-2230 Hours) by 4, corresponding to 3.5 hour periods to represent early morning (0830–1200 Hours); late morning (1200–1530 Hours); early afternoon (1530–1900 Hours) and late afternoon (1900-2230 Hours). The 10 hour (2230-0830 Hours next day) scotophase was considered one treatment.

The experiment was conducted in April and May 2000. Studies were conducted in polystyrene tube cages as described above. One pair (one male and one female) of newly emerged adult wasps was introduced in a polystyrene tube cage. Excess water and honey were provided. During each specific photophase or scotophase treatment, 25 mature adult PPM were provided, in a 28 ml plastic cup (Solo Cup Company, Urbana, IL) to the *A. ananatis* pair. This was done on the day of emergence to provide the parasitoids with parasitization experience. On the second day, twenty-five mealybugs were provided during the same time period as on the first day. Only data from the second day of exposure were used for analysis. Each treatment was replicated 15 times. Numbers of mummified hosts were counted 14 days after parasitization, and analyzed using ANOVA procedures using four preplanned orthogonal contrasts: a) photophase

(0830–2230 Hours) vs. scotophase (2230–0830 Hours next day); b) morning (0830–1530 Hours) vs. afternoon (1530–2230 Hours); c) early morning (0830–1200 Hours) vs. late morning (1200–1530 Hours); and d) early afternoon (1530–1900 Hours) vs. late afternoon (1900–2230 Hours).

*Emergence of adults.* Adult PPM were exposed to about 100 mated *A. ananatis* adult females in 30 cm X 14 cm X 12 cm fine mesh cages. After the mealybugs mummified, 100 mummies were individually transferred to gelatin capsules (Size 2) and held in temperature cabinets at  $24 \pm 1^\circ\text{C}$  and 14:10 (L: D) hours photophase. The study was replicated six times with 100 mummies per replication. Photophase was scheduled to start and terminate at 0800 and 2200 Hours. Parasitoid emergence was recorded at two hour intervals between 0800 and 2200 Hours. Because only one individual emerged between 0000 to 0800 Hours and 2200 to 2400 Hours, the following time blocks were compared: a) 0800 to 1000 Hours (0 to 2 hours of light); b) 1000 to 1200 Hours (2 to 4 hours of light); c) 1200 to 1400 Hours (4 to 6 hours of light), d) 1400 to 1600 Hours (6 to 8 hours of light), e) 1600 to 1800 Hours (8 to 10 hours of light), and f) 1800 to 2400 Hours and 0000 to 0800 Hours (Evening and night).

The adults that emerged during each time period were sexed and counted. The percentages of males and females emerging during each treatment period were calculated. Data were arcsine-square root transformed to normalize the variance distribution and analyzed using ANOVA procedures.

Cumulative emergence percentage data were analyzed by ANOVA procedures to examine if there were any differences between the sexes and among the time of the day.

Nonlinear regression curve fitting was performed for the cumulative emergence data to the following equation following Chapman's method (Sigma Plot 2002).

$$Y = a(1 - e^{-bx})^c \quad [1]$$

where  $a = 100$ ,  $x$  = time after lights turned on,  $b$  and  $c$  are the determinants of the shape of the curve. The time taken for 50% and 90% parasitoid emergence was estimated from the equation.

## RESULTS

### Factors Affecting Parasitoid Longevity

*Honey and water.* Sex, honey and water significantly influenced adult longevity (Table 4.1), but there were no significant interactions ( $P > 0.3$ ) among these factors. Females lived significantly longer ( $F_{1,183} = 9.34$ ,  $P = 0.0026$ ) than males in all the treatments. Access to honey had the most profound effect on parasitoid longevity ( $F_{1,183} = 686.09$ ,  $P < 0.0001$ ). Parasitoids allowed access to honey lived almost six-fold longer than those parasitoids denied honey. Similarly, water increased longevity ( $F_{1,183} = 16.48$ ,  $P < 0.0001$ ).

*Concentration of honey solution.* Parasitoids denied honey lived a mean of only 2.7 days (Table 4.2). Provision of honey significantly prolonged *A. ananatis* longevity compared to water only ( $F_{4,68} = 38.0$ ,  $P < 0.0001$ ). Mean longevities of individuals fed 10% to 50% honey solution were not significantly different, but were significantly less than longevities of those individuals fed pure honey. Pure honey increased mean longevity almost 900 and 200% compared to water only or diluted honey concentrations, respectively.

Table 4. 1. Effects of water and honey on *Anagyrus ananatis* adult longevity.

Treatment	Days $\pm$ SEM <sup>a, b</sup>	
	Female (n = 30)	Male (n = 20)
No honey / water	2.8 $\pm$ 0.13 a	2.2 $\pm$ 0.17 a
Water	3.3 $\pm$ 0.17 a	2.8 $\pm$ 0.15 a
Honey	19.1 $\pm$ 2.09 b	16.5 $\pm$ 2.71 b
Honey + Water	28.3 $\pm$ 2.49 c	26.1 $\pm$ 4.09 b

<sup>a</sup> Data were Log10 transformed and analyzed with ANOVA.

<sup>b</sup> Values within columns followed by the same letter are not significantly different ( $P = 0.05$ ).



Table 4. 2. Impact of honey concentration on adult female *Anagyrus ananatis* longevity.

Percent honey <sup>a</sup>	Days $\pm$ SEM <sup>b, c</sup>
0	2.7 $\pm$ 0.2 a
10	12.3 $\pm$ 1.3 b
25	14.0 $\pm$ 1.5 b
50	12.2 $\pm$ 2.3 b
100	24.6 $\pm$ 3.2 c

<sup>a</sup> Honey was dissolved in water; pure water served as a control.

<sup>b</sup> Data were Log<sub>10</sub> transformed and analyzed with ANOVA.

<sup>c</sup> Values within columns followed by the same letter are not significantly different ( $P = 0.05$ ).

Table 4. 3. Effect of starvation on adult female *Anagyrus ananatis* longevity.

Starvation period (hours)	Individuals surviving starvation		Longevity $\pm$ SEM <sup>a,b</sup>
	Before	After	
0	25	25	25.4 $\pm$ 2.6 a
6	24	24	31.5 $\pm$ 3.1 a
12	24	24	27.4 $\pm$ 2.5 a
24	22	22	28.4 $\pm$ 2 .5 a
48	25	12	18.4 $\pm$ 5.0 b

<sup>a</sup> Data were Log<sub>10</sub> transformed and analyzed with ANOVA.

<sup>b</sup> Values within columns followed by the same letter are not significantly different ( $P = 0.05$ ).

*Starvation.* Starvation significantly impacted parasitoid longevity ( $F_{4,106} = 5.2, P = 0.0007$ ) (Table 4.3). Fifty-two percent (13/25) of the parasitoids died when starved for 48 hours. After being starved for 48 hours, those individuals that survived lived a significantly shorter time (> 30 percent) compared to those that had access to honey within 24 hours of emergence. The parasitoids that had access to honey within 24 hours of emergence survived from 25.4 to 31.5 days (Table 4.3) and did not differ significantly from each other.

*Effect of prior feeding on progeny production.* The post-feeding longevity of the parasitoids was significantly different for the four honey treatments ( $F_{3,30} = 6.97, P = 0.0011$ ) (Table 4.4). Females that were provided honey for only one to two days and then starved lived for a similar length of time after food deprivation as those never provided honey. The parasitoids fed for four days before exposure to the mealybugs survived for a shorter time after starvation than in the other treatments.

The mean number of mummies produced by individual parasitoids during their life time was not affected by the availability of honey before their exposure to mealybug hosts ( $F_{3,30} = 0.67, P = 0.5751$ ). Females denied honey attacked a mean of 3.1 mealybugs whereas females provided honey attacked a mean of 5.8 to 6.2 mealybugs in their lifetime but no statistical differences were observed (Table 4.4). Thus, the provision of honey prior to an augmentative release of *A. ananatis* does not appear to enhance their efficacy in suppressing PPM.

Table 4. 4. Effect of prior feeding on honey on the progeny production and post-feeding longevity of *Anagyrus ananatis*

No. days of access to honey	n	Adult longevity (days)		No. mummies per female	
		Post-feeding <sup>a</sup>	Total	Mean $\pm$ SEM <sup>b</sup>	Range
0	9	2.6 $\pm$ 0.29 a	2.6	3.1 $\pm$ 0.61	1-6
1	9	2.4 $\pm$ 0.18 a	3.4	5.8 $\pm$ 1.48	0-15
2	9	2.1 $\pm$ 0.11 a	4.1	6.2 $\pm$ 1.35	1-12
4	7	1.3 $\pm$ 0.18 b	5.3	6.0 $\pm$ 1.60	0-12

<sup>a</sup> Survival after the parasitoids were deprived of honey as food source. Values followed by same letter within a column are not statistically significant ( $P = 0.05$ ).

<sup>b</sup> Number of mummies per female was log transformed before analysis.

Table 4. 5. Photophase and scotophase influence on rate of *Anagyrus ananatis* oviposition as indicated by adult emergence from parasitized PPM.

Light phase	Time of mealybug exposure (hours)	Corresponding time of the day	Hosts attacked $\pm$ SEM per parasitoid <sup>a,b</sup>
Scotophase	2230–2400, 0000–0830	Night	0.0 $\pm$ 0.0 b
Photophase	0830-1200	Early AM	2.5 $\pm$ 2.3 a
	1200–1530	Late AM	1.5 $\pm$ 2.0 a
	1530–1900	Early PM	3.3 $\pm$ 2.9 a
	1900-2230	Late PM	2.3 $\pm$ 3.2 a

<sup>a</sup> Data were analyzed with ANOVA.

<sup>b</sup> Values within columns followed by the same letter are not significantly different ( $P = 0.05$ ).

## Biological Events

*Ovipositional behavior.* *A. ananatis* attacked PPM during photophase, but did not attack any mealybugs during scotophase ( $F_{1, 56} = 22.55, P < 0.0001$ ) (Table 4.5). The mean number of hosts parasitized ranged from 1.5 to 3.3 hosts per female parasitoid over the photophase intervals examined and were not statistically significant from each other (0500–1200 Hours [Morning] vs. 1200–1900 Hours [Afternoon]:  $F_{1, 56} = 0.97, P = 0.3278$ ; 0500–0830 Hours [early AM] vs. 0830–1200 Hours [late AM]:  $F_{1, 56} = 1.85, P = 0.1794$ ; 1200–1530 Hours [early PM] vs. 1530–1900 Hours [late PM]:  $F_{1, 56} = 1.88, P = 0.1762$ )

*Emergence of adults.* Time of the day had a significant effect on adult emergence ( $F_{5, 55} = 184.2, P < 0.0001$ ) (Table 4.6). Male and female *A. ananatis* adults had similar emergence patterns ( $F_{1, 55} = 0.39, P = 0.5333$ ). Individuals preferred to emerge during photophase, especially in the morning hours. The greatest number of parasitoids emerged within two hours after photophase began and the emergence rate gradually slowed down. The cumulative parasitoid emergence was significantly affected by the time of the day ( $F_{5, 59} = 815.18, P < 0.0001$ ), but there were no differences between the sexes ( $F_{1, 59} = 1.17, P = 0.2848$ ). The data fitted well with the Chapman equation ( $F_{2, 63} = 2271.99, P < 0.0001, R^2 = 0.9859$ ) (Fig 4.1). The equation predicted that 50% emergence would occur within 1.65 hours after light and 90% emergence within 4.65 hours.

Table 4. 6. Adult emergence of *Anagyrus ananatis* during specific time intervals.

Light phase	Interval observed (Hours)	Total light hours	Mean % ± SEM <sup>b, c</sup>	
			Male	Female
Photophase & Scotophase <sup>a</sup>	1800 –0000, 0000–0800	4	0.5 ± 0.5 d	0.0 ± 0 d
Photophase	0800–1000	2	56.6 ± 3.4 a	59.0 ± 1.5 a
	1000–1200	4	27.3 ± 4.1 ab	28.4 ± 1.6 b
	1200–1400	6	10.0 ± 1.9 c	8.3 ± 1.5 c
	1400–1600	8	4.2 ± 1.1 cd	1.9 ± 0.9 d
	1600–1800	10	1.3 ± 0.5 d	2.4 ± 1.5 d

<sup>a</sup> Scotophase began at 2200 Hours

<sup>b</sup> Data were arcine-square root transformed and analyzed with ANOVA.

<sup>c</sup> Values within columns followed by the same letter are not significantly different ( $P = 0.05$ ).

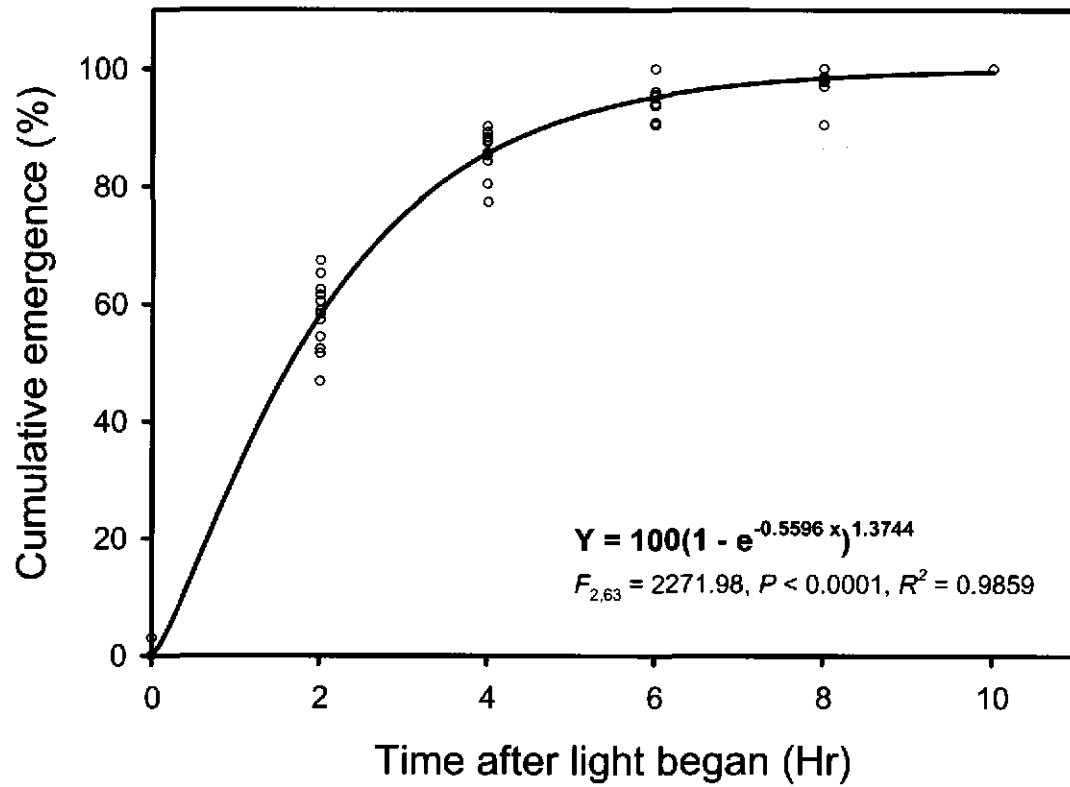


Fig. 4. 1. Adult emergence of *Anagyrus ananatis* during specific time intervals.



## DISCUSSION

The mean longevity of female *A. ananatis* at  $24 \pm 1^\circ\text{C}$  in the studies reported herein ranged from 24 to 31 days when honey and water were provided. Individuals lacking honey died within three days. These results are in agreement with several studies in which parasitoid longevity was greatly increased by the presence of food (Jones and Jackson 1990; Yang *et al.* 1993; McDougall and Mills 1997; Zaviezo and Mills 1999; Sagarra *et al.* 2000). *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae), a parasitoid of diamond back moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), died within three days when starved, but survived for 28 days when provided honey (Yang *et al.* 1993). *Anagyrus kamali* Moursi, a parasitoid of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae), also died within 48 hours when starved, but lived longer when honey was available as food (Sagarra *et al.* 2000). Water alone did not increase *A. ananatis* longevity, indicating that energy reserves in the parasitoid were low and more critical for survival.

*Anagyrus ananatis* fed on pure honey lived longer than those fed with diluted honey though the later also helped *A. ananatis* survive longer than those deprived of all food sources. The shorter longevity of the parasitoids fed on diluted honey solutions compared to those fed on pure honey might have resulted from the consumption of smaller quantities of energy per meal from the diluted honey compared to the pure honey. At the same time, molds developed within a week in the diluted honey, but the pure honey did not develop molds during the experimental period. The molds may have further depleted honey from the solutions making them scarcer. Although *A. ananatis* fed on honey solutions survived significantly longer than the food deprived group, pure

honey was a superior food source. Honey smear on wax paper was provided as food for the mass production of *Aphytis lingnanensis* (Papacek and Smith 1985)

In general, *A. ananatis* females lived longer than males in the study herein. This was contrary to the findings of (Gonzalez-Hernandez 1995). However, current findings are consistent with the longevity of *Anagyrus kamali* females, which always lived longer than males, irrespective of the presence or absence of honey (Sagarra *et al.* 2000). The longevity of *A. ananatis*, which were fed pure honey, was much longer than the previously reported longevity of 9.8 and 10.8 days for females and males, respectively, which were fed on 50% honey solutions (Gonzalez-Hernandez 1995). In the experiments herein, females fed on 50% honey, but without access to suitable mealybug hosts lived for  $12.2 \pm 2.3$  days which is close to the previously reported longevity (9.8 days) (Gonzalez-Hernandez 1995). A reduction in longevity was found in *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), a parasitoid of citrus mealybug, *Planococcus citri* (Risso), (Avidov *et al.* 1967) as well as in *Hyssopus pallidus* (Askew) (Hymenoptera: Eulophidae), a parasitoid of the codling moth (Lepidoptera: Olethreutidae) (Zaviezo and Mills 1999), when they had an opportunity to reproduce. Reduction in longevity and fecundity due to starvation was also reported in a fly parasitoid, *Trichopoda giacomellii* Blanchard (Diptera: Tachinidae) (Coombs 1997). The intrinsic rate of increase was 0.13 and 0.08 whereas the net reproductive rate was 80.3 and 13.4, respectively in the raisin fed flies compared with those fed on water only.

*Anagyrus pseudococci*, a parasitoid of *Planococcus citri*, has been reported not only to survive, but also to have increased fecundity by starving them for four days before providing honey (Islam and Jahan 1992). In contrast, *A. ananatis* individuals need

to feed within 24 hours or many die. *A. ananatis* individuals provided honey for one to four days after adult emergence died within a short time after depriving them of honey (Table 4.4) compared to those continuously provided honey (Tables 1, 2 and 3). It is important to have a continuous supply of honey for *A. ananatis* survival. Similar results were obtained for *Anaphes iole* Girault (Hymenoptera: Mymaridae), an egg parasitoid of *Lygus* spp. Adults with one-hour access to food had a similar fecundity as starved individuals, but those with a continuous supply of honey produced significantly more eggs (Jones and Jackson 1990). *Anagyrus ananatis* that survive starvation of 48 hours or more have a shorter longevity which may be related to increased stress on the parasitoid due to depletion of its food reserves.

Food has long been recognized as a basic factor in determining parasitoid longevity (Stary 1970). Supplemental food is necessary for long-lived and synovigenic parasitoids, but not for short lived and proovigenic parasitoids (Waage *et al.* 1985). *Anagyrus kamali* increased its egg load four days after emergence (Sagarra *et al.* 2000). *Anagyrus pseudococci* (Girault), a primary parasitoid of *Planococcus citri* and the tuber mealybug, *Pseudococcus viburni* (Signoret) (= *Pseudococcus affinis* (Maskell)), were also synovigenic. *A. pseudococci* females had the largest number of eggs ( $25.08 \pm 0.63$ ) in their ovaries five days after eclosion (Islam and Jahan 1992). Water-fed *A. pseudococci* females had about 20% fewer mature eggs than those fed on a 50% honey solution. They exhibited slow egg maturation and rapid egg resorption (Islam and Jahan 1992).

Many hymenopterous parasitoids host feed to supplement their diet with protein needed for ovigenesis (Waage *et al.* 1985), but *A. ananatis* was never observed to host feed.

*Anagyrus ananatis* laid all of its eggs during photophase. Oviposition was not concentrated during any particular time of the day which suggests that mealybug hosts would be attacked any time during daylight. Similar results were also found with *Diadegma semiclaustum*, which preferred to emerge in the early morning, 0600 to 0900 Hours, and oviposited mostly between 0600 to 1800 Hours (Yang *et al.* 1993).

Insects adjust their adult emergence patterns in response to environmental cues. Mexican rice stem borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae), emerges immediately after scotophase begins, and males emerged earlier than females (Spurgeon *et al.* 1995). Hymenopteran parasitoids show positive phototaxis and are active during photophase and inactive during scotophase (Avidov *et al.* 1967; Stary 1970). *Anagyrus ananatis* was not only attracted to light, but also used daylight as a cue for synchronizing emergence from mummies and as a stimulus for host attack. Morning emergence would give them an opportunity to search for food and hosts in the surrounding environment for the remaining daylight hours. Although males and females emerged at similar times of the day, males within a cohort emerged a few days prior to females (Gonzalez-Hernandez 1995). This behavior would guarantee that males will be available for mating when females emerge.

## CONCLUSIONS

Separate provision of honey and water would be beneficial for the mass production of this parasitoid in the laboratory. However, the provision of honey prior to an augmentative release would not be beneficial to the parasitoid and additional carbohydrate sources must be located by *A. ananatis* following release in order to extend

its longevity in the field. Many parasitoids can utilize honeydew as well as the leaf exudates and floral nectars as food sources (Idris and Fraius 1995; Olson and Nechols 1995; Damon *et al.* 1999). Honeydew serves as a source of adult food and a kairomone for detecting insect hosts in many parasitoids (Stary 1970; Heidari and Copland 1993; Singh *et al.* 2000). When present, ants may remove mealybug-produced honeydew before it is accessible to parasitoids. Therefore, *A. ananatis* may have to rely on floral and extra-floral nectaries as carbohydrate sources in the natural environment. Because most commercial pineapple plantings are monocultures, *A. ananatis* may find it difficult to locate and procure food sources. This lack of food may be detrimental for the biological control of PPM. Therefore, it is important to further investigate the natural sources of food for *A. ananatis* in attempts to better conserve natural enemy populations in the field.

[Stary, 1970 #1447] suggested preferentially releasing parasitoids in early morning or late in the evening on bright, sunny days. Early morning field releases would approximate the natural setting of parasitoid emergence, providing an entire day for searching. Late evening releases would give individuals time to acclimate themselves to the crop environment before beginning to search the next morning.

## REFERENCES

- Avidov, Z., Y. Rossler and D. Rosen. 1967. Studies on an Israelei strain of *Anagyrus pseudococci* (Girault) (Hym.: Encyrtidae). II Some biological aspects. *Entomophaga* 12: 111-118.
- Beardsley, J. W., T. H. Su, F. L. McEwen and D. Gerling. 1982. Field investigations on the interrelationships of the big headed ant, the gray mealybug and the pineapple wilt disease in Hawaii. *Proceedings of the Hawaiian Entomological Society* 24: 51-67.
- Beardsley, J. W. 1993. The pineapple mealybug complex: taxonomy, distribution and host relationships. pp. 383-386. *In "Acta Horticulturae 334"* (D. P. Bartholomew and K. G. Rohrbach Ed.). *Proceedings, First International Pineapple Symposium, 2-6 November, 1992 Honolulu, Hawaii.*
- Carter, W. 1932. Pineapple mealybug, *Pseudococcus brevipes* (Ckll), occurring on pineapple plants. *Ecology* 13: 296-304.
- Carter, W. 1945. Some etiological aspects of mealybug wilt. *Phytopathology* 35: 305-315.
- Carter, W. 1963. Mealybug wilt of pineapple: A reappraisal. *Annals of the New York Academy of Sciences* 105: 741-764.
- Collins, J. L. 1960. *The Pineapple: Botany, Cultivation and Utilization*. Leonard Hill, London, 295 pp.

- Coombs, M. T. 1997. Influence of adult food deprivation and body size on fecundity and longevity of *Trichopoda giacomellii*: A South American parasitoid of *Nezara viridula*. *Biological Control* 8: 119-123.
- Damon, A., H. S. Pacheco, J. V. Mora and A. Santiesteban. 1999. Effect of *Euphorbia hirta* nectar and its component sugars, upon the survival of bethylid parasitoids of the coffee berry borer. *Southwestern Entomologist* 24: 49-59.
- Funasaki, G. Y., P. Y. Lai, L. M. Nakamura, J. W. Beardsley and A. K. Ota. 1988. A review of biological control introductions in Hawaii 1890-1985. *Proceedings of the Hawaiian Entomological Society* 28: 105-160.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. *Advances in Disease Vector Research* 9: 241-259.
- Gonzalez-Hernandez, H. 1995. The Status of Biological Control of Pineapple Mealybugs in Hawaii. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Gonzalez-Hernandez, H., M. W. Johnson and N. J. Reimer. 1999a. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15: 145-152.
- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999b. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- Gunasinghe, U. B. and T. L. German. 1986. Association of virus particles with mealybug wilt of pineapple. *Phytopathology* 76: 1073.

- Heidari, M. and M. J. W. Copland. 1993. Honeydew: a food resource or arrestant for the mealybug predator *Cryptolaemus montrouzieri*? *Entomophaga* 38: 63-68.
- Hu, J. S., D. M. Sether and D. E. Ullman. 1996. Detection of pineapple Closterovirus in pineapple plants and mealybugs using monoclonal antibodies. *Plant Pathology* 45: 829-836.
- Hu, J. S., D. M. Sether, X. P. Liu, M. Wang, F. Zee and D. E. Ullman. 1997. Use of a tissue blotting immunoassay to examine the distribution of pineapple closterovirus in Hawaii. *Plant Disease* 81: 1150-1154.
- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.
- Idris, A. B. and E. Frafius. 1995. Wild flowers as nectar source for *Diadegma insularis* (Hymenoptera: Ichneuminidae), a parasitoid of Diamond back moth (Lepidoptera: Yponmeutidae). *Environmental Entomology* 24: 1726-1735.
- Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Islam, K. S. and M. Jahan. 1992. Influence of different types of food on the reproductive performance of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae). *Bangladesh Journal of Entomology* 2: 17-22.
- Jahn, G. C. and J. W. Beardsley. 1998. Presence absence sampling of mealybugs, ants, and major predators in pineapple. *Journal of Plant Protection in the Tropics* 11: 73-79.



- Jones, W. A. and C. G. Jackson. 1990. Mass production of *Anaphes iole* for augmentation against *Lygus hesperus*: effects of food on fecundity and longevity. *Southwestern Entomologist* 15: 463-468.
- McDougall, S. J. and N. J. Mills. 1997. The influence of hosts, temperature and food sources on the longevity of *Trichogramma platneri*. *Entomologia Experimentalis et Applicata* 83: 195-203.
- Noyes, J. S. and M. Hayat. 1994. Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae). Natural History Museum/CAB International, London, 554 pp.
- Olson, D. L. and J. R. Nechols. 1995. Effects of squash leaf trichome exudates and honey on adult feeding, survival, and fecundity of the squash bug (Heteroptera: Coreidae) egg parasitoid *Gryon pennsylvanicum* (Hymenoptera: Scelionidae). *Environmental Entomology* 24: 454-458.
- Papacek, D. F. and D. Smith. 1985. *Aphytis lingnanensis*, pp. 373-381. *In Handbook of Insect Rearing Vol. I* (P. Singh and R. F. Moore Ed.), Elsevier, New York.
- Petty, G. J. and H. Tutsin. 1993. Ant (*Pheidole megacephala* F.) - Mealybug (*Dysmicoccus brevipes* Ckll.) relationship in pineapples in south Africa. pp. 387-395. *In "Acta Horticulturae 334"* (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First international pineapple symposium, 2-6 Nov, 1992 Honolulu, Hawaii.
- Rai, B. K. and A. K. Sinha. 1980. Pineapple: chemical control of mealybug and associated ants in Guyana. *Journal of Economic Entomology* 73: 41-45.

- Reimer, N., J. W. Beardsley and G. Jahn. 1990. Pest ants in the Hawaiian Islands, pp. 40-50. *In Applied Myrmecology. A World Perspective* (K. vander Meer, K. Jaffe and A. Cedeno Ed.), West View Press, Boulder, Colorado.
- Reimer, N. J. and J. W. Beardsley. 1990. Effectiveness of hydramethylnon and fenoxycarb for control of bigheaded ant (Hymenoptera: Formicidae), an ant associated with mealybug wilt of pineapple in Hawaii. *Journal of Economic Entomology* 83: 74-80.
- Reimer, N. J. 1994. Distribution and impact of alien ants in vulnerable Hawaiian ecosystems, pp. 11-22. *In Exotic Ants* (D. F. Williams Ed.), Westview Press, Boulder, Colorado.
- Sagarra, L. A., C. Vincent and R. K. Stewart. 2000. Fecundity and survival of *Anagyrus kamali* (Hymenoptera: Encyrtidae) under different feeding and storage temperature conditions. *European Journal of Entomology* 97: 177-181.
- SAS Institute. 1999. The SAS System for Windows release 8.0. SAS Institute, Cary, NC, USA.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- Sigma Plot. 2002. Sigma Plot 2002 for Windows Version 8. SPSS Inc.
- Singh, R., K. Singh and B. S. Upadhyay. 2000. Honeydew as a food source for an aphid parasitoid *Lipolexis scutellaris* Mackauer (Hymenoptera: Braconidae). *Journal of Advanced Zoology* 21: 77-83.

- Spurgeon, D. W., P. D. Lingren, J. R. Raulston and T. N. Shaver. 1995. Pupal developmental and adult emergence pattern of the mexican rice borer (Lepidoptera: Pyralidae). *Environmental Entomology* 24: 76-79.
- Sary, P. 1970. *Biology of Aphid Parasites (Hymenoptera: Aphididae) with Respect to Integrated Control*. Dr W Junk N.V., The Hague, 651 pp.
- Waage, J. K., K. P. Carl, N. J. Mills and D. J. Greathead. 1985. Rearing entomophagous insects, pp. 45-66. *In Handbook of Insect Rearing Vol. I* (P. Singh and R. F. Moore Ed.), Elsevier, New york.
- Williams, D. J. and G. W. Watson. 1988. *The Scale Insects of Tropical South Pacific Region*. CAB International, Wallingford, 260 pp.
- Yang, J. C., Y. I. Chu and N. S. Talekar. 1993. Biological studies of *Diadegma semiclaussum* (Hym.: Ichneumonidae), a parasite of diamond back moth. *Entomophaga* 38: 579-586.
- Zaviezo, T. and N. Mills. 1999. Aspects of the Biology of *Hyssopus pallidus* (Hymenoptera: Eulophidae), a Parasitoid of the Codling Moth (Lepidoptera: Olethreutidae). *Environmental Entomology* 28: 748-754.

**V. EFFECT OF PINK PINEAPPLE MEALYBUG HOSTS ON THE SIZE AND  
PROGENY PRODUCTION OF *ANAGYRUS ANANATIS* GAHAN  
(HYMENOPTERA: ENCYRTIDAE)**

**INTRODUCTION**

The pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell), and gray pineapple mealybug (GPM), *D. neobrevipes* Beardsley, are the primary vectors of pineapple mealybug wilt associated closterovirus (Sether *et al.* 1998). Together, the virus and the mealybugs can lead to the development of mealybug wilt disease of pineapple (MWP), which is the most important pineapple disease worldwide (Carter 1939, 1951; German *et al.* 1992; Hu *et al.* 1997). PPM was reported as the most common mealybug found on Hawaiian pineapples (Gonzalez-Hernandez *et al.* 1999b). It is also the most common mealybug pest in other pineapple growing areas. Despite the importation and establishment of several natural enemies, only partial success has been achieved in the biological control of PPM in Hawaii and elsewhere due to the interference of ants (Bartlett 1978; Gonzalez-Hernandez *et al.* 1999a).

*Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae) is the most common PPM natural enemy in Hawaii (Gonzalez-Hernandez *et al.* 1999b). It was introduced into Hawaii from Brazil during 1935-1937 (Gahan 1919; Carter 1937; Sakimura 1939). *Anagyrus ananatis* attacks all PPM stages from second instars to mature adults, but prefers adults for oviposition (Gonzalez-Hernandez 1995). It is haplo-diploid and unmated females produce males only. Development of an augmentative biological control program in Hawaii using *A. ananatis* is under consideration (Gonzalez-Hernandez

*et al.* 1999b). Several factors make *A. ananatis* a good choice for augmentation. In addition to being the most common natural enemy attacking PPM in the presence of ants in Hawaii, it has high host specificity and persistence. It has a short developmental time in the laboratory compared to *Nephus bilucernarius* Muslant (Coleoptera: Coccinellidae), the most common coccinellid predator of PPM in Hawaii (Gonzalez-Hernandez 1995). *A. ananatis* produces an average of 27.7 progeny per female during its nearly 10 day adult lifespan (Gonzalez-Hernandez 1995).

Mass production of the target pest is pivotal to most augmentative biological control programs where no artificial diets are available or factitious hosts do not exist (Chambers 1977; Etzel and Legner 1999). Host stage and size affect the quality of the parasitoids produced. Poor quality of released biological control agents can result in the failure of a biological control program (Knipling 1977). The insectary host may influence parasitoid body size, sex ratio, and developmental time (Lampson *et al.* 1996; Heinz 1998). Many parasitoids discriminate and lay more female eggs in larger hosts and more male eggs in smaller hosts (Waage *et al.* 1985; Bokonon-Ganta *et al.* 1995; Heinz 1998; Bernal *et al.* 1999). The number of hosts attacked and host searching ability are among the most important quality parameters of any field-released parasitoid (Visser 1994). Parasitoid size is usually positively correlated with fitness traits such as fecundity and searching ability (Waage *et al.* 1985; Visser 1994; Lampson *et al.* 1996; Coombs 1997).

A preliminary study in which large (> 0.85 mm) and medium (0.60 - 0.85 mm) sized PPM individuals were used to rear *A. ananatis* in the laboratory suggested that *A. ananatis* females ( $1.85 \pm 0.018$  mm in length) that emerged from large mealybugs were

about 23% larger than females ( $1.50 \pm 0.026$  mm in length) from medium-sized hosts. However, the parasitoids reared on large mealybugs were about 12.3% smaller than field-collected females ( $2.11 \pm 0.016$  mm). The male parasitoids ( $0.91 \pm 0.025$  mm) reared from medium-size hosts were smaller than field-collected males ( $1.09 \pm 0.024$  mm), but those reared on large mealybugs were as large as the field collected males.

Studies were conducted to evaluate the effect of mealybug host size on *A. ananatis* size, sex ratio, and fecundity. Because mealybug host size affected parasitoid size, which subsequently affected the parasitoid's reproductive ability, efforts were made to determine the host size required to produce a parasitoid with the previously reported average reproductive ability of  $\geq 28$  lifetime total progeny (Gonzalez-Hernandez 1995).

## MATERIALS AND METHODS

PPM was obtained from a uniparental (female only) population at the Department of Plant and Environmental Protection Sciences, University of Hawaii. *Anagyrus ananatis* was initially collected in October 1998 from parasitized PPM infesting pineapple fruit at Kunia, Oahu, Hawaii (Del Monte Fresh Produce Inc.) and then produced on adult PPM under laboratory conditions.

Unless otherwise stated, all studies described herein were conducted in a temperature cabinet at  $24 \pm 1^\circ\text{C}$ ,  $55 \pm 5\%$  RH and 14:10 (L: D) hours. PPM was mass-produced on Kobocho squash and harvested using methods described in Chapter 3.

### **Influence of Mealybug Size on Parasitoid Size and Sex**

*Parasitized PPM removed from their host plant substrate.* Harvested mealybug individuals were sized using Standard Sieve Numbers 10, 20, 25, 30, 35, 40 and 60.

Sieves size 20 (= 0.85 mm mesh opening), 25 (= 0.71 mm mesh opening), 30 (= 0.6 mm mesh opening), 35 (= 0.5 mm mesh opening), and 40 (= 0.42 mm mesh opening) were categorized as large, medium-large, medium, medium-small, and small, respectively. Standard sieve Number 10 (= 2 mm mesh opening) did not retain any mealybugs whereas the mealybugs that passed through sieve No 40 were too small for parasitization. Sized PPM (n ≈ 100) were exposed to 2 - 3 day-old mated *A. ananatis* adult females (n ≈ 50) for 24 hours in 30 ml plastic containers (Solo Cup Company, Urbana, IL) contained within a fine mesh cage (30 cm x 14 cm x 12 cm). Parasitoids were provided with honey as supplemental food. The mealybugs were then removed from the oviposition cage and incubated in a second cage of identical dimensions in the same temperature cabinet. The process was repeated 10 times.

*Parasitized PPM maintained on their host plant substrate.* A squash infested mostly with mature adult mealybugs (n ≈ 200) was exposed to 2 - 3 day-old, mated *A. ananatis* females (n ≈ 50) for 24 hours within a temperature cabinet. Parasitized PPM individuals were left on the squash until mummies formed.

After two weeks, length and width of the mummified mealybugs were measured under a calibrated microscope and afterwards individually transferred to size 2 gelatin capsules (Eli Lilly Company, Indianapolis, IN). Upon emergence, adult *A. ananatis* were sexed and body length measured. The relative proportions of males and females were calculated for each size category as described above. The effect of mealybug size category on parasitoid size and sex was determined by analysis of variance (ANOVA) and means separated by least squares means test (SAS Institute 1999).

*Anagyrus ananatis* collected from Kunia to initiate the parasitoid culture were preserved in 70% ethanol and measured for comparison. The effect of mealybug size on the body length of the parasitoids was determined by analysis of variance (ANOVA) and means separated using least squares means test.

*Predicting parasitoid sex ratios.* Information collected on all mummies from the above experiments was used for this exercise. A two-sample *t*-test was used to determine if dimensions (length and width) of mummies producing male and female wasps would significantly differ. The mealybug mummy length was divided into seven categories based on the length (< 1.3, 1.3-1.5, 1.5-1.7, 1.7-1.9, 1.9-2.1, 2.1-2.3, and > 2.3 mm long). Number of males and females emerging in each category were analyzed by Chi-square test. The body sizes of both male and female parasitoids from each category were analyzed with ANOVA and means separated by Tukey's studentized range (HSD) test ( $P = 0.05$ ). Logistic regression analysis was conducted to predict parasitoid sex based on mummy length, width, and length: width ratios (SAS Institute 1999).

### **Influence of Parasitoid Size on Progeny Production**

To test the influence of *A. ananatis* adult size on its progeny production, two methods were used to produce the study organisms. First, *A. ananatis* females and males were reared using mealybug hosts categorized as medium (0.6 -0.85 mm) and large (>0.85 mm) size. The mealybugs were harvested from their squash hosts and sized using the sieve methods described above. Mealybugs were exposed to 2 - 3 day-old, mated *A. ananatis* females ( $n \approx 50$ ) for 2 hours in a fine mesh oviposition cage (30 cm x 14 cm x 12 cm) held in a temperature cabinet programmed as described above. After exposure to the parasitoid, the mealybugs were removed from the oviposition cage and held in a



second cage until mummies developed two weeks later. Mummies were counted and transferred to gelatin capsules (size 2) until parasitoid emergence.

To determine progeny production, a newly eclosed female and male *A. ananatis* were placed in a polystyrene tube unit (19 cm X 8.3 cm diameter) fitted with a fine mesh cloth on one side for ventilation and covered with a polystyrene petri dish lid (9.0 cm diameter) on the other. Water was provided with a moist cotton wick throughout the study period. A droplet of honey was provided on a clean glass microscope slide. Twenty-five large PPM adults were provided daily from Day 1 of the study and replaced every 24 hours until the female died. Potentially parasitized PPM individuals were held in a temperature cabinet until mummies developed two weeks after exposure. Mummies were counted and transferred to gelatin capsules (size 2). Upon emergence, the sex of the wasp was noted. This study was replicated 11 and 8 times each with *A. ananatis* females produced from medium size and large size PPM individuals, respectively.

Parasitoids used in the experiment were also produced from PPM individuals allowed to feed following parasitization. Large size PPM individuals feeding on squash were offered to mated *A. ananatis* females for two hours. Following exposure, the mealybugs were held for the development of mummies. Mummies were treated as described above. Emerging adult *A. ananatis* were paired and placed in oviposition units as described above to determine their total progeny production. The same protocols were used to obtain progeny data. This exercise was replicated 7 times.

Body length of each female parasitoid used in the progeny production study was measured under a calibrated stereomicroscope at the end of the experiment.

*Statistical analysis.* Total progeny production values for both sets of parasitoid data (hosts deprived of squash vs. hosts provided with squash) were log-transformed to normalize the residual distribution. Regression analysis was conducted using total progeny (log transformed) as the dependent variable and body length as the independent variable (SAS Institute 1999). Based on this relationship, the average body length of adult female parasitoids required to produce an average of 28 offspring was determined.

*PPM size required to produce parasitoid females  $\geq 28$  offspring.* The above experiment showed that *A. ananatis* females smaller than 1.4 mm length would produce less progeny (< 28 individuals) than previously reported (Gonzalez-Hernandez 1995) as the norm for *A. ananatis*. This experiment was conducted to determine the minimum size of mealybugs required to produce *A. ananatis* females  $\geq 1.4$  mm long. PPM adults retained by sieves No. 20 ('large' category) and 25 ('medium-large' category) were individually weighed on a Mettler AE 163 Balance (Mettler Instruments Company, Hightstown, NJ). Weighed mealybugs were individually placed in a plastic container (30 mm diameter x 5 mm) and transferred to a plastic shoebox (300 x 170 x 80 mm) which held 40 containers, each with an individual PPM. The shoebox was placed in an oviposition cage containing about 50 mated 2 - 3 day-old adult *A. ananatis* females, fed on honey and water. This experiment was repeated four times, exposing a total of 160 mealybugs to the parasitoids. After 24 hours exposure to the parasitoids, the mealybugs were transferred to a second cage for parasitoid development. After two weeks, mummified mealybugs were measured for length and width under a calibrated microscope, transferred to a gelatin capsule (No. 2), and held until emergence. Upon emergence, wasps were sexed and body length recorded.

Effect of mealybug weight on sex allocation by the parasitoid wasp was compared by ANOVA procedures. The length, width and length: width ratios of male and female mummies and the body length of emerged wasps were compared by ANOVA procedures. The relationship between individual PPM weight and the dimensions of the parasitoid wasp emerged therefrom was evaluated with general linear models and required PPM size determined (SAS Institute 1999).

## RESULTS

### **Influence of Host Size on Parasitoid Size and Sex**

Emerged male wasps were significantly smaller than females regardless of the source or size of the mealybug hosts ( $F_{1, 353} = 1872.8, P < 0.0001$ ) (Table 5.1). The mealybug size significantly influenced the size of *A. ananatis* adults ( $F_{6, 353} = 46.1, P < 0.0001$ ). No *A. ananatis* females were produced from small PPM individuals. Females produced from medium-small and medium size PPM individuals were significantly smaller than those produced from medium-large and large size PPM. Likewise, females produced from medium-large PPM were smaller than those produced from large PPM. Females that developed on PPM hosts deprived of their squash host were significantly smaller than those females that developed on PPM individuals that continued to feed on squash or that were collected from the field. In contrast, large and medium-large size males reared from squash deprived PPM hosts were not significantly smaller than those males reared on hosts allowed to feed following parasitization or those collected from the field.

Table 5. 1. Effect of mealybug size and source on adult *Anagyrus ananatis* length.

Mealybug category	Adult length (mm) <sup>a</sup>	
	Female	Male
Small (< 0.5 mm)	–	0.76 ± 0.038 d
Medium-small (0.5 - 0.6 mm)	1.42 ± 0.095 d	0.89 ± 0.019 c
Medium (0.6 - 0.71 mm)	1.54 ± 0.040 d	0.95 ± 0.032 bc
Medium-large (0.71 – 0.85 mm)	1.73 ± 0.021 c	1.01 ± 0.011 ab
Large (> 0.85 mm)	1.86 ± 0.017 b	1.02 ± 0.009 ab
On squash	2.12 ± 0.040 a	1.05 ± 0.043 a
Field collected (standard check)	2.11 ± 0.016 a	1.09 ± 0.025 a

<sup>a</sup> Values followed by the same letter within a column are not significantly different (P = 0.05).

Table 5. 2. Effect of mealybug host size on sex allocation by *Anagyrus ananatis*.

Mealybug category	Female	Male	% Male
Small (< 0.5 mm)	0	7	100
Medium-small (0.5 - 0.6 mm)	7	15	68.1
Medium (0.6 - 0.71 mm)	22	24	52.2
Medium-large (0.71 – 0.85 mm)	51	36	41.4
Large (> 0.85 mm)	170	57	25.1
On squash	32	12	27.3

There was a significant interaction between the mealybug source and the sex of the emerging parasitoid ( $F_{5, 353} = 21.6, P < 0.0001$ ). The reduction in body size was more pronounced in females than males as the PPM host became smaller. *Anagyrus ananatis* females apparently discriminated egg allocation relative to host mealybug size (Table 5.2). Larger mealybugs were allocated more fertilized eggs (= female offspring) while smaller hosts received unfertilized eggs (= male offspring). The sex ratio indicated that medium-large and large size mealybugs would produce more females than males whereas the medium size hosts would produce approximately a 1:1 (M: F) ratio. Medium-small hosts produced more males than females and the smallest host size produced only males ( $\chi^2 = 40.4, df = 4, P < 0.0001$ ) (Table 5.2).

*Predicting parasitoid sex ratios.* A total of 605 mummies were measured, from which 417 female and 188 male parasitoids emerged. Mean length of mummies ( $2.07 \pm 0.013$  mm) producing female *A. ananatis* adults was significantly larger than mummies ( $1.65 \pm 0.018$  mm) producing males ( $t = 18.2, df = 383, P < 0.0001$ ) (Fig. 5.1). An identical trend was found for the mean widths of mummies producing female ( $1.04 \pm 0.008$  mm) vs. male parasitoids ( $0.92 \pm 0.012$  mm) ( $t = 8.4, df = 337, P < 0.0001$ ). Because of the disproportionate decrease in the length of mummies producing males compared to mummies producing females, the length: width ratio for male wasps ( $2.0 \pm 0.007$ ) was significantly smaller than the ratio for females ( $1.82 \pm 0.012$ ) ( $t = 12.5, df = 331, P < 0.0001$ ). The logistic regression model showed that mummy length and width are suitable variables to predict the sex of the emerging parasitoids. The following equation was developed from the logistic regression:

$$\text{Log (odds F)} = -10.6414 + 13.8189 L - 14.5564 W \quad [1]$$

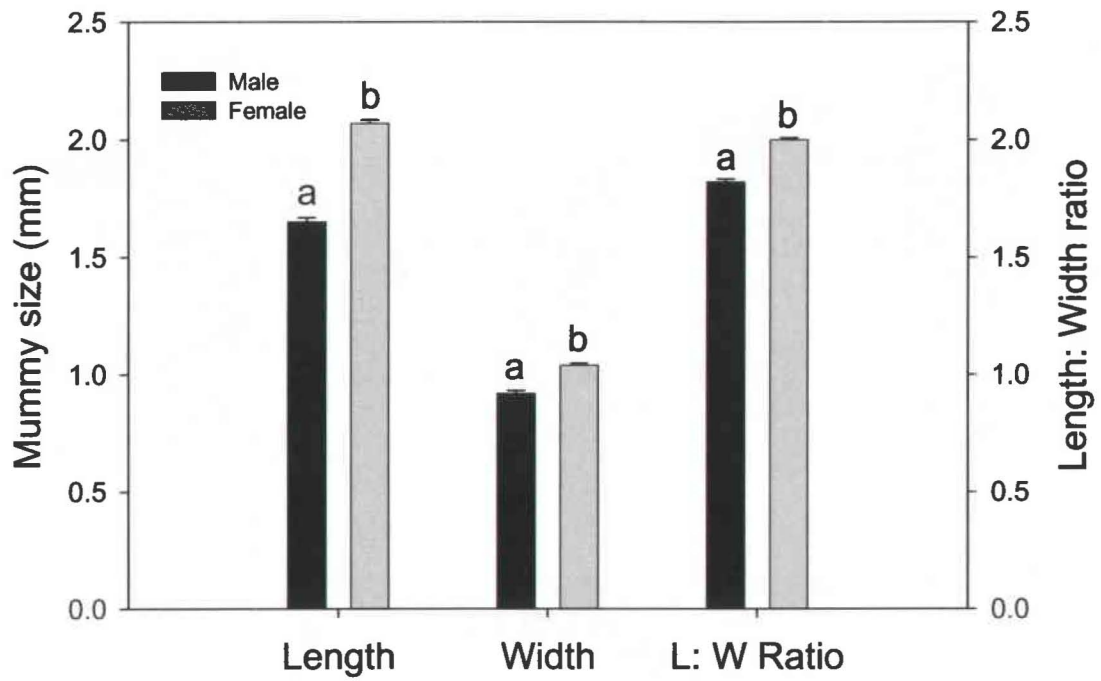


Fig. 5. 1. Mean mummy length, width and their ratios of the mummies producing male and female *A. ananatis*. (Columns with different letters on top are statistically different from each other for each of the parameter measured).

where: odds F is the ratio of the probability ( $P/(1-P)$ ) of a newly emerged parasitoid being female; L is mummy length (mm); and W is mummy width (mm). The probability of the emergence of a female wasp from a given mummy increased as the length of the mealybug mummy increased ( $\chi^2 = 131.1$ ,  $df = 1$ ,  $P < 0.0001$ ), but the probability of female emergence decreased as the width of the mummy increased ( $\chi^2 = 67.8$ ,  $df = 1$ ,  $P < 0.0001$ ). Further analysis indicated that all mummies smaller than 1.3 mm in length produced males and the mummies smaller than 1.7 mm long produced highly male-biased ( $> 2/3^{\text{rd}}$  male) progeny (Table 5.3). Mummies larger than 1.7 mm produced highly female-biased ( $< 1/3^{\text{rd}}$  male) sex ratios, only 1% of the mummies larger than 2.3 mm produced males ( $\chi^2 = 238$ ,  $df = 6$ ,  $P < 0.0001$ ) (Fig. 5.2). Mummy size had a larger influence on the size of the emerging female parasitoids than the males. Mean female body size of the parasitoids produced from the six mealybug mummy categories were significantly different from each other, larger mummies always producing larger female parasitoids ( $F_{5,408} = 209.7$ ,  $P < 0.0001$ ) (Table 5.3). However, the male parasitoids emerging from all the mummies larger than 1.7 mm long were not significantly different from each other. The smallest male progeny (0.73 mm) produced from mummies  $< 1.3$  mm as well as the male progeny produced from 1.3-1.5 mm mummies (0.89 mm) were significantly different from the males produced from rest of the mummy categories ( $F_{6,181} = 36.5$ ,  $P < 0.0001$ ) (Table 5.3).

#### **Influence of Parasitoid Size on Progeny Production.**

Regression analysis of body length of laboratory reared parasitoids on non-feeding hosts showed a significant effect on their ability to reproduce ( $F_{1,17} = 10.75$ ,  $P = 0.0044$ ,  $R^2 = 0.3873$ ), with larger parasitoids producing more progeny (Fig. 5.3). These



Table 5. 3 Relationship of PPM mummy size to the sex and body length of emerged *Anagyrus ananatis*.

Mummy length (mm)	Number <sup>a</sup>		Body length (mm) <sup>b</sup>	
	Female	Male	Female	Male
< 1.3	0	12	--	0.73 ± 0.03 a
1.3 - 1.5	5	38	1.16 ± 0.04 a	0.89 ± 0.01 b
1.5 - 1.7	30	67	1.44 ± 0.03 b	1.00 ± 0.01 c
1.7 - 1.9	69	35	1.58 ± 0.02 c	1.03 ± 0.01 cd
1.9 - 2.1	125	29	1.73 ± 0.01d	1.04 ± 0.01 cd
2.1 - 2.3	105	6	1.88 ± 0.01 e	1.12 ± 0.04 d
> 2.3	83	1	2.07 ± 0.02 f	1.00 cd

<sup>a</sup> Total numbers of females and males were 417 and 188, respectively.

<sup>b</sup> Values followed by the same letter within a column are not significantly different (P = 0.05).

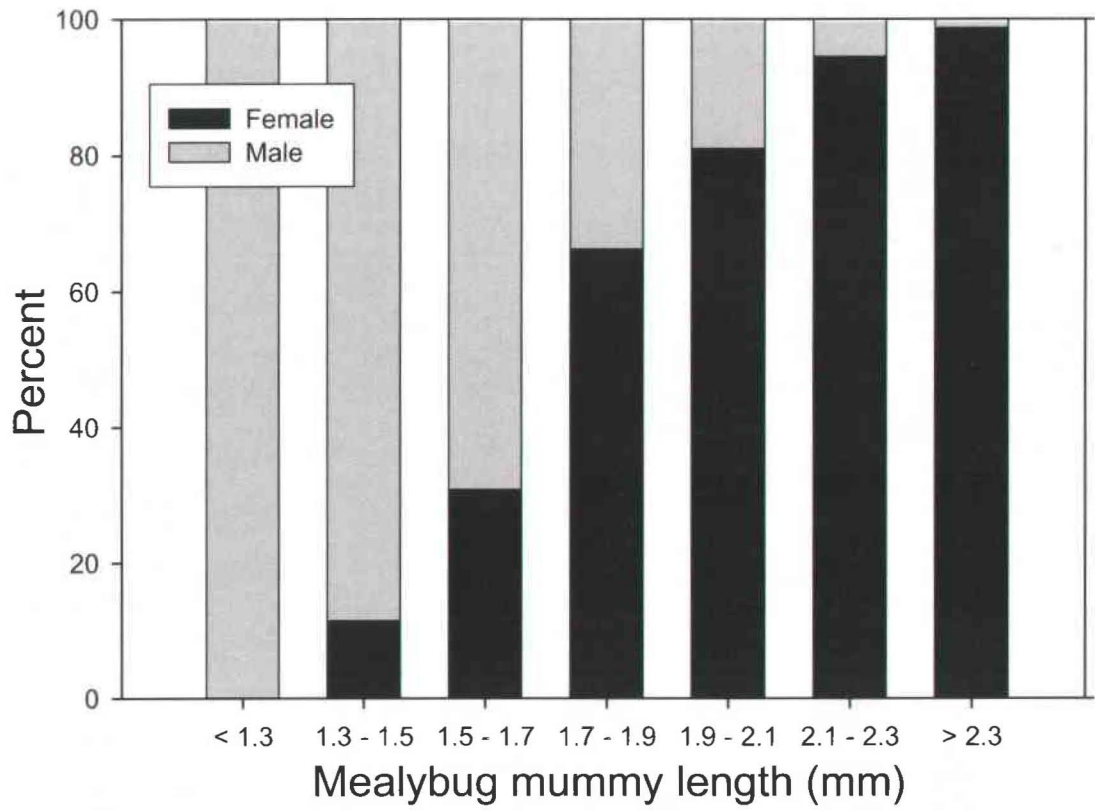


Fig. 5. 2. Effect of Mealybug mummy size on sex allocation by *Anagyrus ananatis*.

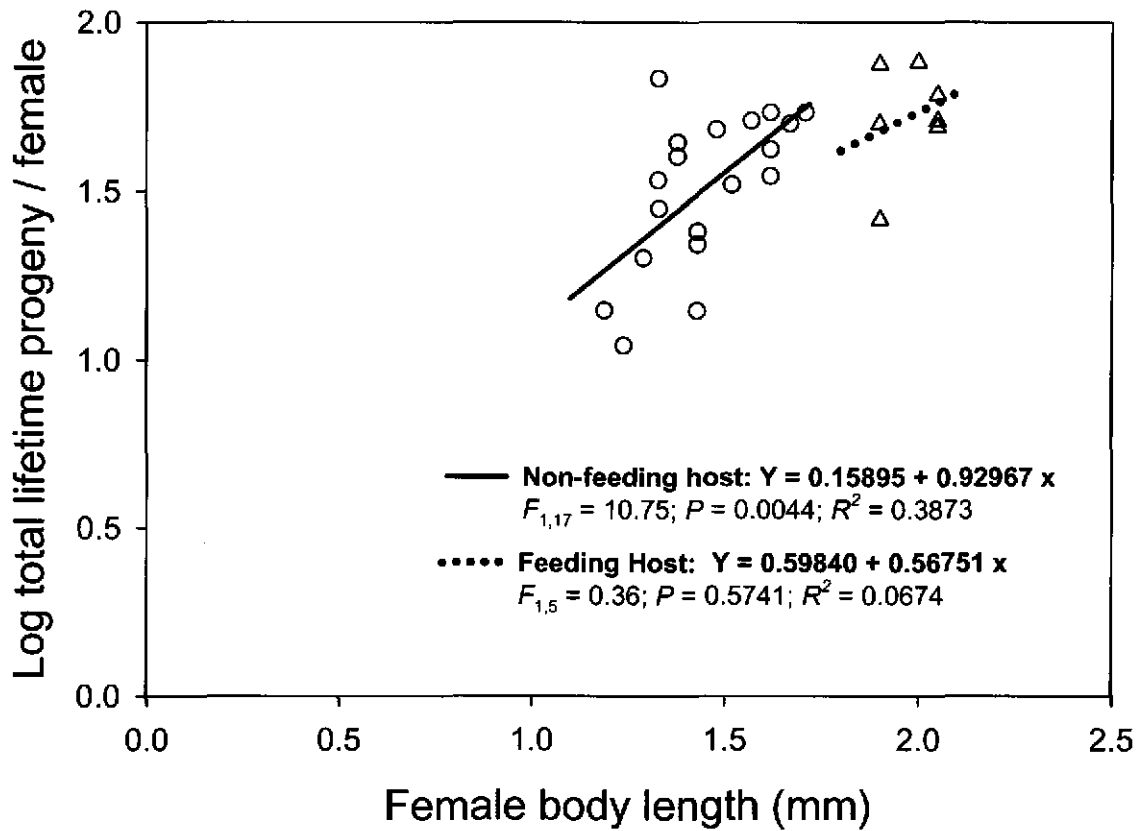


Fig. 5. 3. Relationship between female *A. ananatis* body length and progeny production.

parasitoids had a mean body length of  $1.45 \pm 0.035$  mm (minimum of 1.19 mm to maximum of 1.71 mm). However, no significant effect of body length was demonstrated for parasitoids that were produced on PPM hosts allowed to feed after being parasitized ( $F_{1,5} = 0.36, P = 0.5741, R^2 = 0.0637$ ).

The parasitoids produced from feeding hosts had a mean body length of  $1.98 \pm 0.027$  mm (minimum = 1.90 mm and maximum = 2.05 mm). Based on these results, the body length of an adult *A. ananatis*  $\geq 1.4$  mm would produce a mean progeny of  $\geq 28$  individuals.

*PPM size required to produce parasitoid females  $\geq 1.4$  mm long.* Out of 160 mealybugs exposed to *A. ananatis* females 120 were parasitized. Adult parasitoid emergence occurred from 115 of these mummies, producing 99 female and 16 male adults. PPM individuals attacked by *A. ananatis* ranged from 0.5 to 3.6 mg in weight. The mean weight of hosts ( $1.99 \pm 0.07$  mg) that produced female wasps was significantly greater than that of hosts ( $1.47 \pm 0.19$  mg) that produced males ( $F_{1, 113} = 7.0, P = 0.0093$ ). The mean length of mummies ( $2.11 \pm 0.02$  mm) producing females was significantly longer than those producing males ( $1.77 \pm 0.04$  mm) ( $F_{1, 113} = 34.9, P < 0.0001$ ). Similarly, the mean width of female and male mummies differed significantly ( $F_{1, 113} = 5.4, P = 0.0219$ ). The ratio of the length to width was significantly smaller for male mummies (1.733: 1) compared to that of female mummies (1.915: 1) ( $F_{1, 113} = 30.91, P < 0.0001$ ). Mummy width was highly correlated with the weight of the parasitized mealybug ( $r = 0.822, n = 120, P < 0.0001$ ). Similarly, mummy length was highly correlated with the weight of the parasitized host ( $r = 0.797, n = 120, P < 0.0001$ ). The sex of an emerging parasitoid had a strong influence on its body length. All females

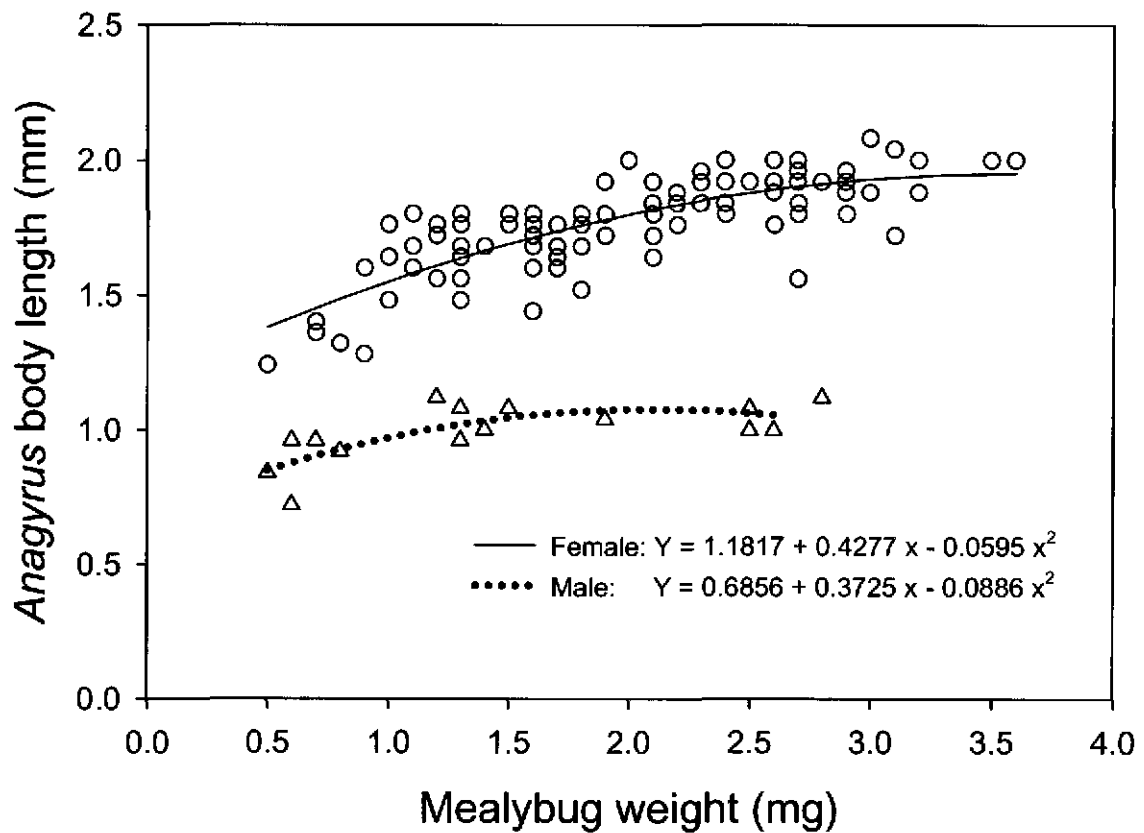


Fig. 5. 4. Relationship between PPM host weight and *A. ananatis* body length produced therefrom.

[Parasitoid sex (S):  $F = 10.7$ ,  $P = 0.0014$ ; PPM weight (W):  $F = 15.5$ ,  $P = 0.0001$ ;  $W^2$ :  $F = 6.3$ ,  $P = 0.0139$ ; S x W:  $F = 0.1$ ,  $P = 0.7858$ ; S x  $W^2$ :  $F = 0.2$ ,  $P = 0.6237$ ;  $df$  for all tests = 1,109].

( $1.77 \pm 0.018$  mm) were larger than the males ( $0.99 \pm 0.026$  mm), regardless of the size of the mealybug host upon which they developed ( $F_{1,113} = 293.48$ ,  $P < 0.0001$ ). Regression analysis showed a significant relationship between mealybug host weight and the length of the emerged parasitoid adult ( $F_{4,110} = 230.8$ ,  $P < 0.0001$ ,  $R^2 = 0.8935$ ) (Fig. 5.4). The intercept for the body length (0.74 mm) of the male was significantly smaller than for the female (1.17 mm) ( $t = 10.59$ ,  $df = 109$ ,  $P < 0.0001$ ), which implied that males were inherently smaller than females. The rate of increase in the body length of the *A. ananatis* was significantly affected by the weight of mealybug ( $F_{1,110} = 26.78$ ,  $P < 0.0001$ ), with a higher rate of increase for females (0.441 mm) than males (0.288 mm) per each-milligram increase in mealybug weight ( $t = 3.76$ ,  $df = 110$ ,  $P = 0.0003$ ). The body size of *A. ananatis* was also significantly affected by a quadratic function (-0.0627) of host weight ( $F_{1,110} = 11.46$ ,  $P = 0.001$ ) (Fig. 5.4). As the weight of the mealybug host increased, there was a decline in the increase rate in *Anagyrus* body length. The rate of decline, however, was not significantly different for the two sexes. Based on these results, mealybugs weighing  $\geq 0.6$  mg would produce female *A. ananatis* with an average reproductive ability comparable to the previously reported ~28 progeny during its life time.

## DISCUSSION

*Anagyrus ananatis* was able to discriminate the size of its mealybug host and preferentially deposit fertilized eggs in the larger hosts and unfertilized eggs in smaller ones. The finding of a female biased sex ratio in larger hosts and male biased sex ratio in smaller hosts was in accord with previous studies (Boavida *et al.* 1995; Bokonon-Ganta

*et al.* 1995; Islam and Copland 1997; Bernal *et al.* 1999). Gonzalez-Hernandez (1995) reported that *A. ananatis* preferred to oviposit in adult mealybugs compared to second or third instars.

A strong positive correlation between mealybug weight and mummy size was not surprising, and it was also expected that larger mummies would produce larger parasitoids. However, the greater impact of host size on *A. ananatis* females may be attributed to the inherent difference in body size of females and males (the latter being about half the size of the former). Male parasitoids would not be as food limited as females when the mealybug host is small. In fact, it was observed that when male *A. ananatis* developed in large PPM individuals, a considerable part of the mealybug body remained unutilized. In *Leptomastix epona* (Walker) (Hymenoptera: Encyrtidae), a solitary endoparasitoid of *Pseudococcus viburni* (Signoret) (Homoptera: Pseudococcidae), the size of both males and females increased with an increase in the size of its host (Karamaouna and Copland 2000). Similarly, the size of females and males of *Metaphycus helvolus* (Compere) (Hymenoptera: Encyrtidae) on black scale, *Saissetia oleae* (Olivier) (Homoptera: Coccidae), increased with an increase in host size (Karamaouna and Copland 2000). Larger hosts produced larger *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae) (Heinz 1998). In contrast, the size of the mealybug host did not affect the size of individuals of the gregarious parasitoid *Pseudaphycus flavidulus* (Brethes) (Karamaouna and Copland 2000). This may be due to an adjustment of clutch size by ovipositing females. Contrary to the findings reported here, in *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug, *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae), male

body size increased with an increase in the host size, but female body size was independent of the host size at oviposition (Bokonon-Ganta *et al.* 1995). However, the latter study was consistent with the results herein relative to the finding that female wasps emerging from any host instar were always larger than the corresponding males.

Although allowing mealybugs to continue feeding on squash following parasitization produced larger parasitoids, it is more efficient to harvest and size mealybugs for *A. ananatis* propagation. Total parasitization in any large parasitoid production system is an unobtainable goal. Unparasitized mealybugs (mostly early instars as well as some adults) continue to produce honeydew, which serves as a media for mold and mite development, resulting in contamination of the parasitoid culture. Although the effect of mite infestations on the quality of *A. ananatis* has not been quantified, it is thought to be detrimental based on casual observation and experience. Infestation of honey bee pupae with the mite *Varroa jacobsoni* Oudemans (Mesostigmata: Verroidae) caused significant mortality and other adverse effects on honey bees (Brodsgaard *et al.* 2000).

A major objective of mass rearing protocols for augmentative biological control is to provide methods that maximize the production of high quality female parasitoids (Luck *et al.* 1999). Body size is a prominent quality parameter of laboratory-reared arthropods (Legner 1988). The findings reported herein indicated that despite the ability of *A. ananatis* to parasitize all PPM stages from the second instar to adult females, gravid mature adult female hosts would produce large parasitoids with high fecundity, even when parasitoids were produced on PPM hosts removed from their plant food source. With the increase in the body length of *A. ananatis*, there was also a corresponding



increase in the width of the head, thorax, and abdomen. The larger abdomen size likely contributes to the logarithmic relationship between body length and the fecundity of the parasitoid. The narrow range of parasitoid body length (and abdomen size) and small sample size of *A. ananatis* reared on feeding hosts failed to detect the relationship. Because harvesting and sizing of mealybugs for parasitoid production is the preferred method of rearing, producing adequately large sized parasitoids is of prime concern.

Like *A. ananatis*, other mealybug parasitoids also attack young as well as adult mealybugs, but host size affected sex allocation. *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae), a parasitoid of mango mealybug, reproduced on first, second and third instar nymphs (Boavida *et al.* 1995). The proportion of male offspring was lowest when *R. invadens* individuals were parasitized as mature adult females and the proportion increased with decreasing host size (Bokonon-Ganta *et al.* 1995).

*Apoanagyrus* (= *Epidinocarsis*) *lopezi* DeSantis (Hymenoptera: Encyrtidae), a parasitoid of *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), mainly used small hosts for the production of male offspring, while larger hosts produced female-biased sex ratios (Dijken *et al.* 1991). In *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae), a parasitoid of cassava mealybug, *Phenacoccus herreni* Cox & Williams (Homoptera: Pseudococcidae), parasitism was highest on third instar hosts in no-choice tests, while in choice tests, parasitism was high in third instars and adults (Bertschy *et al.* 2000). In *Metaphycus flavus* (Howard) and *M. stanleyi* Compere (Hymenoptera: Encyrtidae) offspring sex ratios were controlled by host size; larger hosts led to more female progeny (Bernal *et al.* 1999). Similar results were obtained with *Catolaccus grandis* (Hymenoptera: Pteromalidae), a parasitoid of the cotton boll weevil,

*Anthonomus grandis* Boheman (Coleoptera: Curculionidae), where larger hosts produced more female biased sex ratios (Heinz 1998).

*Anagyrus ananatis* can determine between the size of the host while depositing eggs, and lay more fertilized eggs than unfertilized eggs on larger hosts, and more unfertilized eggs than fertilized eggs on smaller hosts. Production of mealybugs by infesting Kobocho squash with mature adults (Chapter 3) and use of sieves was found useful to size the mealybugs. When harvested mealybugs were left for two hours on the stacks of the standard sieves, the standard sieve No. 20 (=0.85 mm opening) retained most of the gravid adult female mealybugs with an average weight of 1.69 mg; standard sieve No. 25 (=0.71 mm opening) held mature adult female mealybugs with an average weight of 0.74 mg; and standard sieve No. 30 (=0.60 mm opening) retained mostly young adult females and some younger instar mealybugs with an average weight of 0.54 mg. Use of either an intermediate sieve size ( $> 0.6$  mm and  $< 0.71$  mm) or shorter time ( $< 2$  hours) with sieve pore size 0.71 mm may help retain mealybugs of  $\geq 0.6$  mg weight which are suitable for parasitoid production. Use of selected mealybugs would encourage the production of adequately large sized female-biased progeny, making the production system more efficient. Mealybugs  $< 0.6$  mg would not be suitable hosts for parasitoid rearing, but may be used for re-infesting squash for mealybug production. Infesting new squash with these mealybugs would produce mature gravid females within 3 - 4 weeks (see Chapter 3), which could then be used for parasitoid production. The harvested mealybugs should be exposed to the parasitoids as quickly as possible so that the mealybugs are still healthy and unstressed.

The ability to predict the sex of a parasitoid before its emergence would be helpful to estimate the number of females from a given number of mummies being used for field release. Mummies that were relatively longer and had an elliptical shape (mean length: width ratio = 2.0: 1) produced females, whereas the mummies shorter in length and apparently round shape (mean length:width ratio = 1.8: 1) produced males. A similar relationship was reported in *Aphidius rhopalosiphi* de Stefani-Perez (Hymenoptera: Ichneumonidae), which attacks the cereal aphid *Metopolophium dirhodum* (Walker) (Homoptera: Aphididae). Oval mummies produced females and round mummies produced male wasps (Jan *et al.* 1996).

#### CONCLUSIONS

Female *A. ananatis* body size was a major contributing factor in determining the reproductive ability of parasitoids reared from unfed hosts. It is important to produce adequately large-sized female parasitoids to achieve the desired biological control of PPM. The use of PPM adults as hosts for producing *A. ananatis* should produce larger females with a more female-biased sex ratios than the use of younger stages. Mealybugs larger than 0.6 mg in weight should be used for parasitoid rearing. The size of PPM mummies was a good predictor of the sex and body size of the parasitoid contained within, and may be used as an important indicator of parasitoid quality in a mass production system.

## REFERENCES

- Bartlett, B. R. 1978. Pseudococcidae, pp. 137-171. *In* Introduced Parasites and Predators of Arthropod Pest and Weeds: A World Review (C. P. Clausen Ed.), U. S. Department of Agriculture, Handbook No. 480, Washington D.C.
- Bernal, J. S., R. F. Luck and J. G. Morse. 1999. Host influences on sex ratio, longevity, and egg load of two *Metaphycus* species parasitic on soft scales: Implications for insectary rearing. *Entomologia Experimentalis et Applicata* 92: 191-204.
- Bertschy, C., T. C. J. Turlings, A. Bellotti and S. Dorn. 2000. Host stage preference and sex allocation in *Aenasius vexans*, an encyrtid parasitoid of the cassava mealybug. *Entomologia Experimentalis et Applicata* 95: 283-291.
- Boavida, C., M. Ahounou, M. Vos, P. Neuenschwander, J. J. M. V. Alphen and J. J. M. van Alphen. 1995. Host stage selection and sex allocation by *Gyransoidea tebygi* (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug, *Rastrococcus invadens* (Homoptera: Pseudococcidae). *Biological Control* 5: 487-496.
- Bokonon-Ganta, A. H., P. Neuenschwander, J. J. M. V. Alphen, M. Vos and J. J. M. van Alphen. 1995. Host stage selection and sex allocation by *Anagyrus mangicola* (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug, *Rastrococcus invadens* (Homoptera: Pseudococcidae). *Biological Control* 5: 479-486.
- Brodsgaard, C. J., W. Ritter, H. Hansen and H. F. Brodsgaard. 2000. Interactions among *Varroa jacobsoni* mites, acute paralysis virus, and *Paenibacillus* larvae and their influence on mortality of larval honeybees in vitro. *Apidologie* 31: 543-554.

- Carter, W. 1937. Importation and laboratory breeding of two chalcid parasites of *Pseudococcus brevipes* (Ckl.). *Journal of Economic Entomology* 30: 370-372.
- Carter, W. 1939. Injuries to plants caused by insect toxins. *Botanical Review* 5: 273-326.
- Carter, W. 1951. The feeding sequence of *Pseudococcus brevipes* (Ckll) in relation to mealybug wilt of pineapple in Hawaii. *Phytopathology* 41: 769-780.
- Chambers, D. L. 1977. Quality control in mass rearing. *Annual Review of Entomology* 22: 289-308.
- Coombs, M. T. 1997. Influence of adult food deprivation and body size on fecundity and longevity of *Trichopoda giacomellii*: A South American parasitoid of *Nezara viridula*. *Biological Control* 8: 119-123.
- Dijken, M. J. V., P. Neuenschwander, J. J. M. V. Alphen and W. N. O. Hammond. 1991. Sex ratios in field populations of *Epidinocarsis lopezi*, an exotic parasitoid of the cassava mealybug, in Africa. *Ecological Entomology* 16: 233-240.
- Etzel, L. K. and E. F. Legner. 1999. Culture and colonization, pp. 125-198. *In Handbook of Biological Control: Principles and Applications of Biological Control* (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.
- Gahan, A. B. 1919. Identity of the *Anagyrus* that parasitizes the pineapple mealybug (Hymenoptera: Calchidoidea: Encyrtidae). *Proceedings of the Hawaiian Entomological Society* 8: 357-360.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. *Advances in Disease Vector Research* 9: 241-259.
- Gonzalez-Hernandez, H. 1995. The Status of Biological Control of Pineapple Mealybugs in Hawaii. Ph.D Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.

- Gonzalez-Hernandez, H., M. W. Johnson and N. J. Reimer. 1999a. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15: 145-152.
- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999b. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- Heinz, K. M. 1998. Host size-dependent sex allocation behaviour in a parasitoid: Implications for *Catolaccus grandis* (Hymenoptera: Pteromalidae) mass rearing programs. *Bulletin of Entomological Research* 88: 37-45.
- Hu, J. S., D. M. Sether, X. P. Liu, M. Wang, F. Zee and D. E. Ullman. 1997. Use of a tissue blotting immunoassay to examine the distribution of pineapple closterovirus in Hawaii. *Plant Disease* 81: 1150-1154.
- Islam, K. S. and M. J. W. Copland. 1997. Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. *Biocontrol Science and Technology* 7: 449-456.
- Jan, S., H. F. van-Emden and Z. U. Nurullahoglu. 1996. What sex is your mummy? (Homoptera: Aphididae). *Entomologist* 115: 185-190.
- Karamaouna, F. and M. J. W. Copland. 2000. Host suitability, quality and host size preference of *Leptomastix epona* and *Pseudaphycus flavidulus*, two endoparasitoids of the mealybug *Pseudococcus viburni*, and host size effect on parasitoid sex ratio and clutch size. *Entomologia Experimentalis et Applicata* 96: 149-158.

- Knipling, E. F. 1977. The theoretical basis for augmentation of natural enemy, pp. 79-123. *In* Biological Control by Augmentation of Natural Enemies (R. L. Ridgeway and S. B. Vinson Ed.), Plenum Press, New York.
- Lampson, L. J., J. G. Morse and R. F. Luck. 1996. Host selection, sex allocation, and host feeding by *Metaphycus helvolus* (Hymenoptera: Encyrtidae) on *Saissetia oleae* (Homoptera: Coccidae) and its effect on parasitoid size, sex and quality. *Environmental Entomology* 25: 283-294.
- Legner, E. F. 1988. Quantitation of heterotic behavior in parasitic Hymenoptera. *Annals of the Entomological Society of America* 81: 657-681.
- Luck, R. F., L. Nunney and R. Stouthamer. 1999. Sex ratio and quality in the culturing of parasitic Hymenoptera: a genetic and evolutionary perspective, pp. 653-672. *In* Handbook of Biological Control: Principles and Applications of Biological Control (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.
- SAS Institute. 1999. The SAS System for Windows release 8.0. SAS Institute, Cary, NC, USA.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by the experiment station of the pineapple producers cooperative association during the period 1930-1938. *Proceedings of the Hawaiian Entomological Society* 10: 352.

Visser, M. 1994. The importance of being large: the relationship between size and fitness of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology* 63: 963-978.

Waage, J. K., K. P. Carl, N. J. Mills and D. J. Greathead. 1985. Rearing entomophagous insects, pp. 45-66. *In Handbook of Insect Rearing Vol. I* (P. Singh and R. F. Moore Ed.), Elsevier, New York.



## VI. DEVELOPMENT OF *ANAGYRUS ANANATIS* GAHAN (HYMENOPTERA: ENCYRTIDAE) AT CONSTANT TEMPERATURES

### INTRODUCTION

Pink pineapple mealybug, PPM, *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae), is one of the most widely distributed, tropicopolitan mealybug species in the world (Williams and Watson 1988). PPM and the gray pineapple mealybug (GPM), *Dysmicoccus neobrevipes* Beardsley, can efficiently transmit pineapple mealybug wilt associated virus and induce mealybug wilt of pineapple (Sether *et al.* 1998), which is the major insect-transmitted disease of pineapple worldwide. Both mealybug species are believed to have originated in South America where pineapple also originated (Collins 1960; Bartlett 1978; Rohrbach *et al.* 1988; Beardsley 1993). These mealybugs were accidentally introduced into Hawaii in the early 1900's (Kotinsky 1910; Pemberton 1964). During the 1930's, more than 20 natural enemies of *D. brevipes* were introduced to Hawaii from different parts of the world (Swezey 1939; Funasaki *et al.* 1988; Gonzalez-Hernandez 1995). A field survey on the Hawaiian Islands of Oahu and Maui revealed that at least five exotic species of natural enemies of PPM have successfully established in pineapple plantings. Among these species, *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae) was the most prevalent PPM natural enemy (Gonzalez-Hernandez *et al.* 1999b). It was introduced into Hawaii between 1935-37 from Brazil (Carter 1937) and is highly specific. PPM is the only recorded host in Hawaii, although other hosts established in Hawaii are reported to be attacked by the parasitoid in other localities (i.e., *Antonina graminis* (Maskell) from Brazil (DeSantis

1980), *Ferrisia virgata* (Cockerell) from South America (DeSantis 1979), *Planococcus citri* (Risso) from Uruguay (DeSantis, 1964) (see Noyes and Hayat 1994). Unfortunately, the presence of mealybug-attending ants, especially the bigheaded ant, *Pheidole megacephala* F. (Hymenoptera: Formicidae), greatly interferes with the activities of predators and parasitoids of mealybugs in pineapple (Gonzalez-Hernandez *et al.* 1999a).

The organophosphate diazinon is the only pesticide registered for use against mealybugs on pineapple in Hawaii (HDOA 2001). The possibility of developing an augmentative biological control program for PPM control was suggested by Gonzalez-Hernandez *et al.* (1999b). *Anagyrus ananatis* was identified as a prime natural enemy candidate for augmentation based on its prevalence, host specificity and persistence (Gonzalez-Hernandez 1995). *Anagyrus ananatis* attacks all the stages of PPM except the crawlers, completes its developmental cycle in about 23 days at 26°C, and produces an average of 27.7 offspring during a 10 day adult life span (Gonzalez-Hernandez 1995). However, adequate biological information about this parasitoid for the purposes of mass production was lacking. Knowledge of the developmental biology and temperature requirements of a parasitoid are necessary for designing efficient mass production techniques, determining safe protocols for cold storage of mass-reared parasitoids, and predicting seasonal occurrence, population dynamics and distribution of populations.

The time required to complete development to the reproductive stage of poikilothermic organisms, such as insects, is shorter at high temperatures than at low temperatures (Andrewartha and Birch 1954). For these organisms, the rate of change of development is linear over a range of favorable temperatures (Andrewartha and Birch 1954) with some deviations from linearity at both the lower and upper extremes. The

range of temperatures when the development rate is linear is termed the optimal temperature range (Ikemoto and Takai 2000). The parameter “total accumulated temperature” (K) is viewed as a biological constant, which remains constant for an insect species under a given set of environmental conditions (Davidson 1944).

Several approaches have been used to model the developmental rates of insects (Briere *et al.* 1999 and references therein). The linear model, which uses the inverse function of the time required to complete development, is one of the most commonly used models due to its simplicity and ability to predict an acceptable lower developmental threshold ( $T_o$ ) and an accumulated effective temperature (K) requirement in field situations where extreme temperatures are rare (Briere *et al.* 1999). However, care must be taken to ensure that the insect hosts used in the developmental studies are similar to those utilized in the field (Campbell *et al.* 1974). Campbell *et al.* (1974) also concluded that the developmental rate was linear for a range of temperatures and that the commonly encountered temperatures under field conditions would lie almost exclusively on the straight-line section. The major problem with this conventional linear model is that the value of  $T_o$  is found by extrapolation and therefore estimated inaccurately (Campbell *et al.* 1974). Ikemoto and Takai (2000) pointed out three problems with the conventional linear model: a) the difficulty in accurately detecting the critical temperatures, b) lower estimation of  $T_o$  and a higher estimation of K and c) failure to take into account the error in the temperature measurements, which leads to lower estimation of the slope of the regression line.

The objectives of this study were to: a) determine the lower developmental threshold and identify the optimum temperature for rearing *A. ananatis* using the

conventional methodology and that of Ikemoto and Takai (2000); and b) determine the length and characteristics of the developmental stages of *A. ananatis* at a constant temperature.

## MATERIALS AND METHODS

PPM individuals used in this experiment were obtained from cultures maintained at the Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, Hawaii. Mature Kobocho squash were infested with uniparental adults of PPM and covered in vermiculite, to facilitate removal of honeydew produced by PPM, within a paper carton and incubated at  $23 \pm 1^\circ\text{C}$ . The infested fruit was removed from the carton each week, the vermiculite was agitated and the infested fruit replaced and covered with the vermiculite. After about eight weeks, the mealybugs were harvested from the squash, sized using standard sieves, and mealybugs  $\geq 0.6$  mm were exposed to *A. ananatis* for parasitization (for more details see Chapter 3). *Anagyrus ananatis* were initially collected from parasitized PPM mummies on pineapple at Kunia, Oahu, Hawaii, during October 1998. They were further propagated using adult PPM.

For both studies described below, newly parasitized mealybugs were obtained in the following manner. Mature leaves from greenhouse grown pineapple plants were cut into lengthwise sections about 10 cm long with the tips removed. Cut section ends were dipped in molten wax. After the wax cooled, 50 mature adult mealybugs were transferred with a fine brush onto each leaf section. About 100 female and 50 male adult *A. ananatis* were transferred to each of four fine-mesh 'organza' (Fabric Mart, Honolulu, HI) (about 36 openings/cm) cages (30 cm x 14 cm x 12 cm). The cages were provisioned with

honey and water and placed within a temperature cabinet at  $23.8 \pm 0.5^\circ\text{C}$  and  $50 \pm 5\%$  RH. Ten PPM-infested leaf sections were introduced to each of the cages containing *A. ananatis* for two hours exposure. Afterwards, the parasitized mealybugs on leaf sections were removed from the cages and used in the studies below.

### **Developmental Threshold**

Five temperature regimes (T) were used in the study:  $14.6 \pm 0.2$ ,  $19 \pm 0.3$ ,  $23.8 \pm 0.5$ ,  $28.9 \pm 0.3$  and  $31 \pm 0.3^\circ\text{C}$  (Mean  $\pm$  SD). For each temperature, a clear 53 L (40 cm x 55 cm x 33 cm) plastic container (Clear Stack on Wheels™, IRIS USA INC, Pleasant Prairie, WI) with a snap tight lid was used as an experimental chamber and placed within the temperature cabinet. Humidity was maintained at  $72 \pm 5\%$  RH with a saturated salt solution (Lide 1993). Light was provided by 20 W fluorescent tubes for a 14:10 (L: D) hour cycle. Parasitized mealybugs on leaf sections (as described above) were transferred to a 150 mm diameter petri dish and placed on a wire mesh (5 cm x 2.5 cm) support. Temperature and relative humidity of each chamber were continuously monitored with a Dickson TH Trace TL120 Data Logger every 15 minutes throughout the experimental period and mean temperature within each chamber was calculated.

Leaf sections were checked daily for mummified mealybugs. Mummies were transferred to 5 ml glass test tubes (Disposable Culture Tubes, Fisher Scientific, Pittsburgh, PA) plugged with cotton and held in the same chamber. Mummies were checked daily at 24 hour intervals from the time of parasitization for adult emergence and sexed. Days (D) required for each parasitoid to complete development from egg to adult were recorded. Rate of parasitoid development (r) was calculated by  $r = 1/D$ , and multiplied by 100 to convert to percent development per day (Campbell *et al.* 1974).

*Statistical analysis.* Days required to reach adulthood (D) and the rate of development (r) of male and female parasitoids among the various temperature regimes (T) were submitted to general linear model procedures (Proc GLM) (SAS Institute 1999). Increases in developmental rate ( $\Delta r$ ) for each 1°C increase in temperature for each temperature interval between the temperature regimes used in the experiment were calculated by:

$$\Delta r = (r_2 - r_1)(t_2 - t_1)^{-1} \quad [1]$$

where  $\Delta r$  = increase in the rate of development for increase in each 1°C;  $r_1$  = rate of development at lower temperature ( $t_1$ ); and  $r_2$  = rate of development at a higher temperature ( $t_2$ ). The association of the rearing temperature with the days taken to complete development from egg to adult and the development rate were estimated by correlation analysis (Kishore *et al.* 1994).

The lower developmental threshold ( $T_0$ ) and accumulated effective temperature (K) were estimated by least square linear regression procedures following two methods. First, the conventional method was used (Briere *et al.* 1999, and references therein).

A Generalized Linear Model was fitted with Proc GLM (SAS Institute 2000) to describe the developmental rate (r) with temperature, sex and their interactions as independent variables (Campbell *et al.* 1974). The observations for the lowest temperature ( $14.6 \pm 0.2^\circ\text{C}$ ) were omitted (Campbell *et al.* 1974) and the analysis was performed again because this temperature was close to the threshold temperature estimated ( $\cong 12^\circ\text{C}$ ) by this model. The data were reanalyzed without the interaction term (leaving only the two main effects: temperature and sex because the interaction term

between sex and incubation temperature was not statistically significant). From the regression equation:

$$Y = a + bx \quad [2]$$

where Y is the developmental rate at temperature x, and a and b are the intercept and rate of development, respectively. Based on the regression equation,  $T_o$  ( $= -a/b$ ) and K ( $= 1/b$ ) were derived. The standard errors for the estimated parameters were not calculated due to the complexity and poor accuracy (Campbell *et al.* 1974; Ikemoto and Takai 2000).

The second method used to estimate  $T_o$  and K was based on the work of Ikemoto and Takai (2000). A new variable, DT, was calculated by multiplying the days (D) taken to complete the developmental cycle and the incubation temperature (T). The variable DT was submitted to general linear model (Proc GLM) with sex, D, and their interaction as the independent variables (SAS Institute 1999).

Analysis was performed using all data points available, and then by omitting those data points near the lower threshold (as explained for the conventional method). A simple regression analysis also indicated that all data points from the rearing temperature of 14.6°C greatly influenced the slope of the regression line and were therefore excluded from the analysis. Further analysis was performed only with the two main effects (sex and days to emergence, D) in the model because the interaction effect between sex and D was not statistically significant. The advantage of this model was that the intercept in the equation directly provided the value for K and the slope provided  $T_o$ . The standard errors of the parameters were calculated.

The optimum temperature range was estimated by reanalyzing the data by excluding one or both extreme temperatures used in the study (Ikemoto and Takai 2000)

to achieve the best linear fit of the model. Also, the increases in the rate of development as well as the developmental rate were taken into consideration.

### **Biological Development of *Anagyrus ananatis***

Pineapple leaf sections with parasitized mealybugs were prepared as described above, and placed in a fine mesh organza cage, as described earlier, that was held in a temperature cabinet at  $23.5 \pm 0.5^{\circ}\text{C}$  and  $70 \pm 5\%$  RH (as recorded every 15 minutes with a Dickson TH Trace TL120 Data Logger). Light was provided by 20 W fluorescent tubes on a 14:10 (L: D) hour cycle.

At experiment initiation (Day 0) and everyday thereafter until Day 10, about 30 randomly selected mealybugs were preserved daily in 70% ethyl alcohol. By Day 11, all remaining parasitized mealybugs began to mummify, and they were transferred to 20 ml glass vials (20 mummies per vial). On each following day, 20 mummies (1 vial) were preserved in 70% ethyl alcohol until all the remaining mummies developed into adults (Day 26). Preserved mealybugs were carefully dissected at 25x magnification to observe the parasitoid stage, pertinent characteristics, and / or structures developed.

The count data on the stages of wasps observed on each day was converted to percentage data based on the total number of parasitoids observed on that day after parasitization. Orientation of the heads of the wasp larvae and pupae was noted for each mummy relative to the mealybug host, analyzed by Chi-square ( $\chi^2$ ) goodness of fit test and expressed as the percentage of the total number observed.



## RESULTS

### Developmental Threshold

Thirty-eight to 52 individuals completed their development to the adult stage at each temperature regime, except 14.6°C at which only 8 females and 1 male completed development (Table 6.1). The developmental rate was highly correlated with rearing temperature ( $r = 0.9819$ ,  $n = 188$ ,  $P < 0.0001$ ) and the days required to complete development were negatively correlated ( $r = -0.8307$ ,  $n = 188$ ,  $P < 0.0001$ ) with an increase in temperature. Time to complete development decreased significantly with an increase in rearing temperature ( $F_{1,185} = 410.84$ ,  $P < 0.0001$ ). The mean time to complete development was 107.9 days at 14.6°C, but only 15.6 days at 31°C (Fig. 6.1). There were no significant differences in development times between males and females due to rearing temperature ( $F_{1,185} = 2.13$ ,  $P = 0.1457$ ) (Fig. 6.1). There was a linear increase in the developmental rate from 14.6 to 28.9°C, but as the rearing temperature further increased the developmental rate plateaued, thereby suggesting that 31°C may be near the higher threshold temperature (Fig. 6.2).

*Lower development threshold and total heat requirement.* The values estimated for  $T_0$  by the conventional and Ikemoto and Takai methods (ca. 12°C) were near the lowest temperature regime (14.6°C) in the study, thus data from the 14.6°C regime were excluded from the final analysis (Table 6.2). Using the conventional analysis, there was no significant interaction ( $F_{1,175} = 0.06$ ,  $P = 0.812$ ) between sex and rearing temperature relative to developmental rate. Developmental rate increased linearly with an increase in rearing temperature ( $F_{1,176} = 3893.76$ ,  $P < 0.0001$ ). Males developed faster than females ( $F_{1,176} = 9.43$ ,  $P = 0.0025$ ) (Fig. 6.3). The value for K was estimated as  $288 \pm 0.2$  Degree

Table 6. 1. Number of adult *Anagyrus ananatis* that completed development from egg to adult at various temperatures.

Temperature (°C) Mean ± SD	Female	Male	Total
14.6 ± 0.2	8	1	9
19.0 ± 0.3	36	8	44
23.8 ± 0.5	23	15	38
28.9 ± 0.3	34	11	45
31.0 ± 0.3	38	14	52

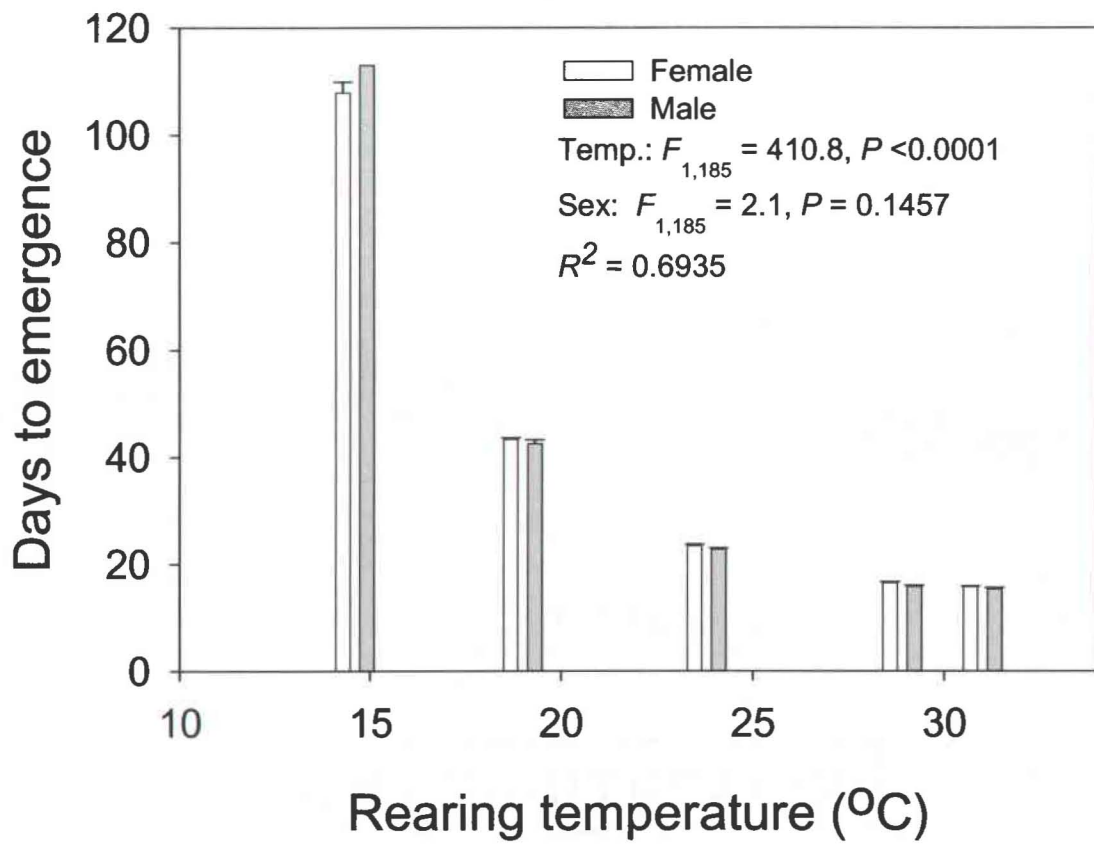


Fig. 6. 1. Effect of rearing temperature on the time to complete development to adult stage of *A. ananatis*.

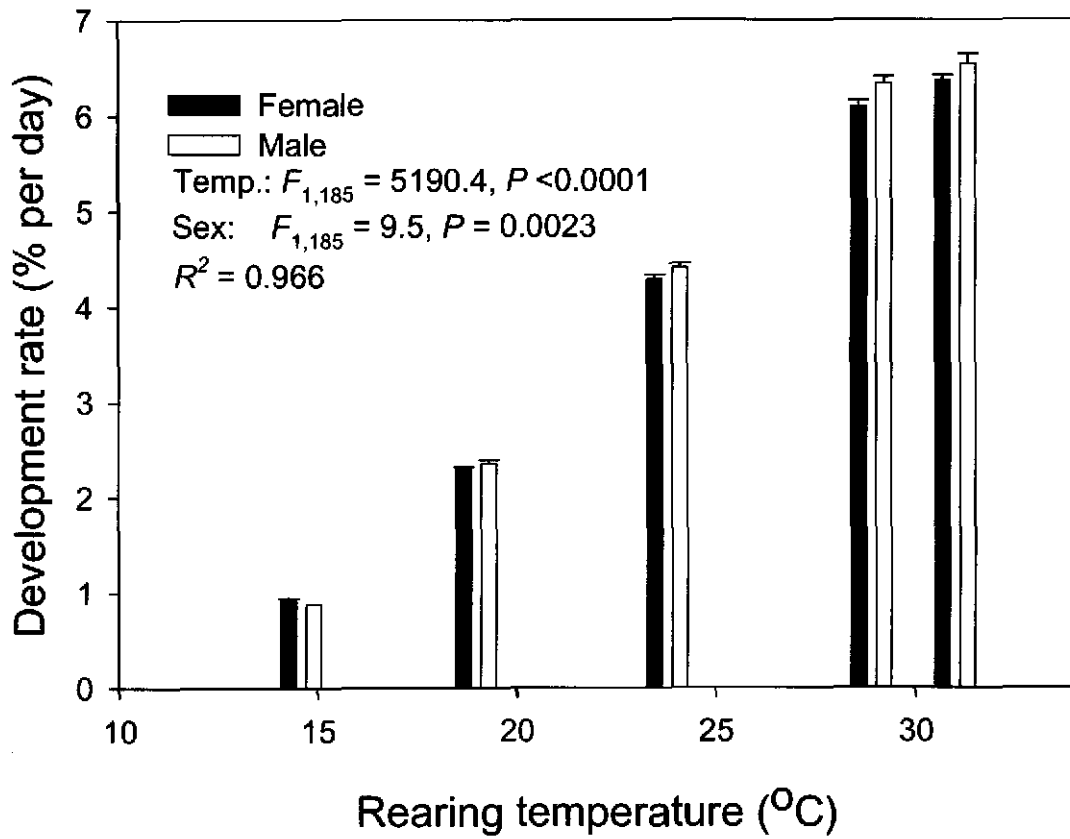


Fig. 6. 2. Effect of temperature on the developmental rate of *A. ananatis*

Table 6. 2. The lower temperature threshold, ( $T_o$ ) in °C and accumulated temperature requirement (K) in degree days (DD) of *Anagyrus ananatis* as determined by the conventional method and that described by Ikemoto and Takai (2000) using all data collected and without data from the 14.6°C temperature regime.

Sex	All observations			Without 14.6°C data		
	n	$T_o \pm \text{SEM}$ (°C)	$K \pm \text{SEM}$ (DD)	n	$T_o \pm \text{SEM}$ (°C)	$K \pm \text{SEM}$ (DD)
Conventional method						
Male	49	11.50	288.3	48	11.49	288.2
Female	139	12.00	288.3	131	12.01	288.2
Ikemoto & Takai Method						
Male	49	$12.12 \pm 0.07$	$277.2 \pm 3.2$	48	$12.65 \pm 0.12$	$265.3 \pm 3.7$
Female	139	$12.12 \pm 0.07$	$286.9 \pm 2.6$	131	$12.65 \pm 0.12$	$274.9 \pm 3.4$

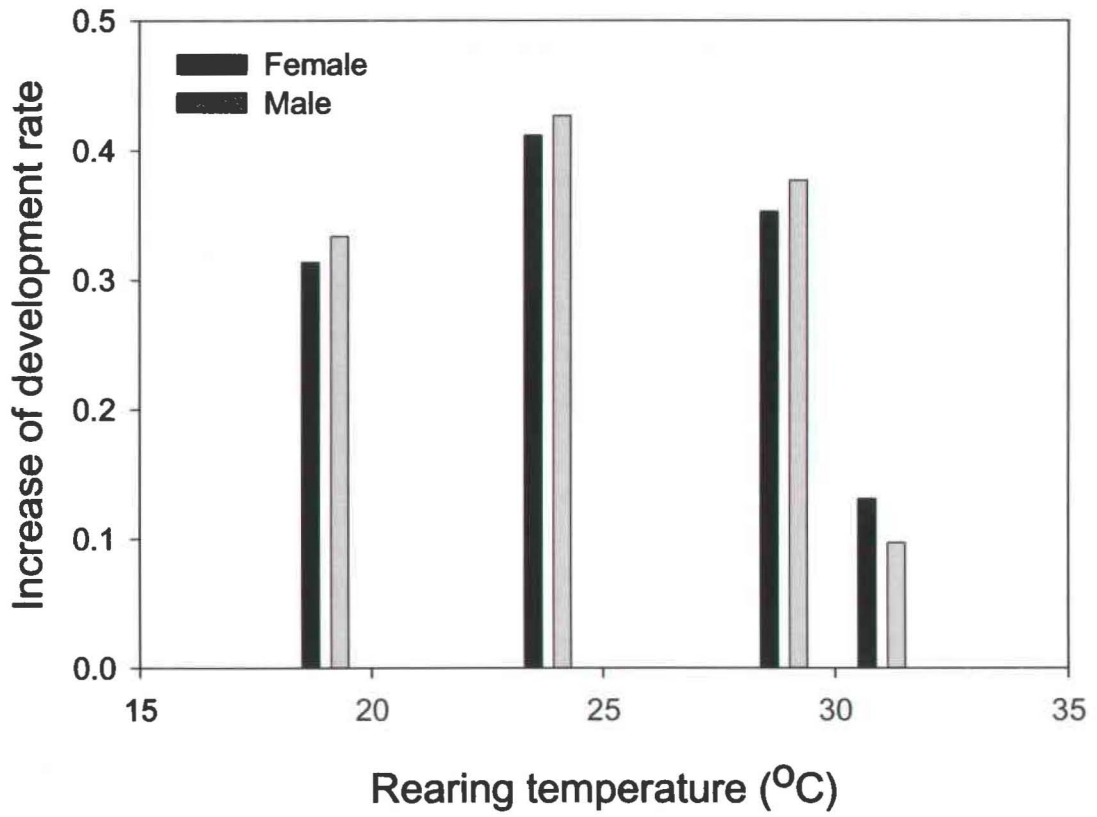


Fig. 6. 3. Change in the developmental rate of *A. ananatis* in relation to the increase in the rearing temperature.

Days (DD) for both males and females, but the estimated value for  $T_o$  for males was lower (11.49°C) than for females (12.01°C) (Table 6.2). Standard errors for the estimated values were not calculated because of the possible inaccuracies and difficulties (Campbell *et al.* 1974).

Using the Ikemoto and Takai method, there was no significant interaction between sex and the developmental time (D) ( $F_{1,175} = 0.06, P = 0.8011$ ). Reanalysis of data with only the main factors (sex and developmental time) showed that both factors were highly significant (Sex:  $F_{1,176} = 9.77, P = 0.0021$ ; Developmental time:  $F_{1,176} = 11,036.0, P < 0.0001$ ). This method estimated a single value for  $T_o$  for both sexes (12.65 ± 0.12°C), but estimated a lower K value for the males (265.3 ± 3.7 DD) compared to the females (274.9 ± 3.4 DD) ( $F_{1,176} = 9.77, P = 0.0021$ ).

*Optimum temperature.* The developmental rate closely followed a linear model from 19 to 28.9°C, but deviated from linearity at 31 and 14.6°C. The change in developmental rate per unit increase in rearing temperature was also similar (> 0.3 % per day per °C) within this temperature range (19 - 28.9°C) (Fig.6.3). However, the increase in development rate declined sharply (ca. 0.1 % per day per °C) as rearing temperature increased from 28.9 to 31°C. Thus, 19 - 29°C may be considered the optimum temperature range for laboratory rearing of *A. ananatis*. The highest increase in developmental rate (0.427%) per °C increase in the rearing temperature was found between 19 and 23.8°C (Fig. 6.3), suggesting that the optimum rearing temperature was around 24°C.

### **Biological Development of *Anagyrus ananatis***

The mean temperature during the experimental period was  $23.5 \pm 0.5^{\circ}\text{C}$  leading to a mean daily accumulation of 10.85 DD of heat above  $T_o$  ( $12.65^{\circ}\text{C}$ ). The results were graphically presented as percent of development stages relative to heat accumulation.

*Eggs.* Eggs were inserted through the dorsal surface of the PPM body, mostly in the posterior half, although a few were laid in the anterior half as well. Eggs were encyrtiform as described by [Clausen, 1940 #585]. They were laid among the host's egg mass and attached to the host's cuticle. The eggs were creamy white in color, almost elliptical in shape measuring  $0.344 \pm 0.025$  mm in length and  $0.273 \pm 0.015$  mm in width. They had a short slender stalk ( $0.094 \pm 0.008$  mm) with a slightly swollen apex attached to the host cuticle. An aeroscopic plate stretched almost the entire length of the egg. From the third day after parasitization, a dark, brown scar was observed on the mealybug cuticle at the point of ovipositor insertion, but the egg stalk did not protrude from the host's body surface as suggested by Clausen (1940). Parasitoid larvae were found attached at this scar until the mealybug mummified.

*First instar.* The eggs hatched on Days 3-4, after at least 22 DD accumulated (Fig. 6.4). When 44 DD were accumulated, all eggs had hatched. No eggs remained unhatched on Day 5 after parasitization. Newly eclosed larvae were partially enclosed in the egg chorion. The head capsule was distinctly visible with a relatively large and well-developed pair of mandibles. The thoracic region of the larvae also exhibited slightly protruded, poorly developed legs. The body of a newly hatched larva was generally translucent, but appeared slightly orange in the gut area, probably due to the consumption of host eggs. As many as five, first-instar individuals were observed within one host



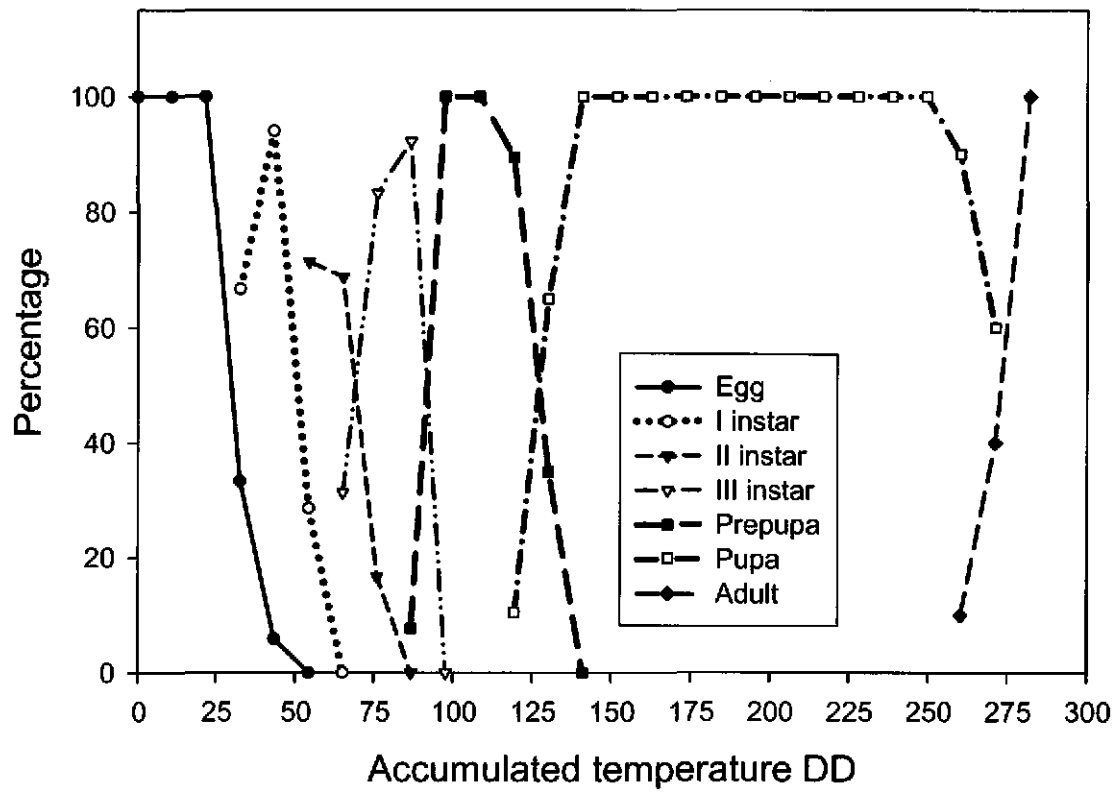


Fig. 6. 4. Developmental stages of *A. ananatis* at constant temperature of  $23.5 \pm 0.5^\circ\text{C}$

mealybug (i.e., superparasitization), but only one survived to maturity. On a few occasions both eggs and first instars were observed in the same mealybug host. First instar larvae were observed from Day 3 to Day 5. It was the shortest larval stage, lasting only one to two days. The first instar measured  $0.63 \pm 0.06$  mm long and  $0.17 \pm 0.15$  mm wide (n = 22).

*Second instar.* The second instars were observed from Day 4 to Day 7 after oviposition (Fig. 6.4) and measured  $1.28 \pm 0.93$  mm long and  $0.30 \pm 0.03$  mm wide (n = 13), with a distinct orange colored gut. The larvae remained attached to the host cuticle, but the head capsule was less distinct. The thoracic segments had leg-like structures. At this stage, when more than one larva was present, only one larva dominated within a mealybug. Dark scars, especially on the thoracic region, were observed on the less developed parasitoid larvae, and were presumed to have been inflicted by their competitor.

*Third instar.* These immatures were “C” shaped, fat and fleshy, and translucent except within the central part of the body, which was largely orange-colored. They were observed from Day 6 until Day 8 after oviposition (Fig. 6.4). The head was little developed, thorax was highly enlarged; and posterior (i.e., abdominal) region was slender. The larvae measured  $2.03 \pm 0.13$  mm long and  $0.78 \pm 0.06$  mm wide (n = 21). Exuviae were observed covering part of the posterior region of the larva. The larvae were highly fragile and easily damaged while dissecting. The point of attachment between the host and the parasitoid was quite weak, and easily broken while being handled. When the mealybugs were carefully dissected from the ventral surface, it was possible to observe the intact connection.

Larval development from oviposition was completed when the parasitoid accumulated about 87 DD. When the larvae were fully developed, a large part of the host tissue had been consumed and the mealybug was dead. The host skin slightly folded and shriveled before the mealybugs transformed into mummies. The head of the larva was commonly (ca. 82 %) oriented to the anterior end of the mealybug host, but some (ca. 18 %) larvae were found oriented to the posterior end of the host ( $\chi^2 = 3.0$ ,  $df=1$ ,  $P = 0.083$ ). Larval orientation was dependent on the site of oviposition in the host mealybug. When oviposition occurred in the posterior end of the host body, the larval head was directed to the head of the mealybug and *vice versa*.

*Prepupae.* The fully developed larvae stopped feeding and detached from their stalks prior to entering the prepupal stage,. The prepupae were beige in color and were observed from Day 8 to Day 12 after oviposition (Fig 6.4). They measured  $2.33 \pm 0.12$  mm long and  $1.00 \pm 0.05$  mm wide ( $n = 17$ ). About 62% of the prepupae were oriented with their head towards the anterior of the mealybug at the time of dissection ( $\chi^2 = 3.3.1$ ,  $df=1$ ,  $P = 0.069$ ). The mealybug appeared bloated and football shaped, with a hard surface exhibiting a smooth and shiny interior surface. The mummy cuticle wall was probably reinforced with other mealybug parts that were not consumed and some secretion from the parasitoid larvae. The prepupae were highly fragile and could easily be damaged while opening the hardened mummy.

*Pupae.* Deposition of meconial pellets and metamorphosis to the pupal stage began on the Day 11 with the accumulation of 122 DD, and completed by Day 13 with the accumulation of 144 DD (Fig. 6.4). Pupae were shiny white in color, exarate with well-developed appendages and wings. Three body regions were clearly differentiated by

Day 14. About 85% of the pupae had their heads orientated toward the mealybug posterior, and meconia were deposited within the head region of the mealybug ( $\chi^2 = 115.28$ ,  $df=1$ ,  $P < 0.0001$ ). This corresponds closely to the proportion of larvae that oriented towards the head region ( $\chi^2 = 0.86$ ,  $df=1$ ,  $P = 0.354$ ). The compound eyes and the ocelli turned pink on Day 14, after accumulating 166 DD, and afterwards gradually became darker. A pale greenish triangular area was observed on the abdominal region of the pupae. Later, dark pigmentation appeared on the abdominal and thoracic regions. The males developed a more uniform black color whereas the females had light brown bands.

*Adults.* First adult emergence occurred 24 days after parasitization (after accumulating 259 DD). By Day 26, all the mummies had produced adults with the accumulation of 281 DD. These results fit very well with the temperature developmental study, where the mean DD required to complete development was 271 DD (Fig. 6.4).

## DISCUSSION

The estimated parameters of  $T_0$  and  $K$  generated by the conventional methodology and that of Ikemoto and Takai (2000) were similar. It may be noted that the conventional method always estimated the  $T_0$  for the males lower than that for the females, whereas the Ikemoto and Takai method predicted a higher  $T_0$  for males (12.71°C) than females (12.63°C) (when interaction effects were included in the analysis). A slightly higher  $T_0$  was reported for males (13.06°C) of a closely related species, *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae), a parasitoid of citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae), compared to its

females (12.57°C) (Islam and Jahan 1993). In the current study, the proportion of males completing development at 14.6°C was much lower than the proportions at the higher experimental temperatures. This may have resulted from higher mortality of males at the colder temperature because of the higher  $T_o$  for males. In cool temperature storage studies (see Chapter 7), there were more adverse effects on the male parasitoids than females.

The Ikemoto and Takai method estimated a slightly higher  $T_o$  than the conventional method, but was adjusted by predicting a lower DD requirement. It also estimated a lower K for males, by about 10 DD, suggesting that males emerge about one to two days earlier than females reared at about 25°C. These results are in line with a previous study where the males and females completed their development in 21.2 and 23.3 days, respectively, at  $26 \pm 2^\circ\text{C}$  (Gonzalez-Hernandez 1995).

The  $T_o$  value (12.65°C) estimated for *A. ananatis* in this study was also supported by a study evaluating the effects of cool temperature storage on *A. ananatis* adults when held in storage inside mealybug mummies (see Chapter 7). *Anagyrus ananatis* individuals within mealybug mummies stored at 10.1°C for 2 to 4 weeks required a similar post-storage development period to emerge as those without cold storage (control), suggesting that they did not develop any further while stored. On the other hand, individuals within mummies stored at 14.6°C required a shorter incubation period compared to the control after they were transferred to a rearing temperature of 24°C. These findings indicated that the  $T_o$  value falls between 10.0 and 14.6°C. The  $T_o$  of 12.65°C was within the range of those values for other mealybug parasitoids such as *Leptomastix dactylopii* Howard (13.15°C), *Anagyrus pseudococci* (Girault) (12.81°C)

and *Leptomastidae abnormis* (Girault) (10.28°C) attacking *Planococcus citri* (Tingle and Copland 1988). Insect species of tropical origin generally have higher  $T_o$  values (12.9 - 13.7°C) than subtropical (10.4 - 11.1°C) and temperate species (7.8 - 9.3°C) (Honek 1996). The  $T_o$  would also be affected by the environment in which insects are collected due to their adaptation to the local environment (Campbell *et al.* 1974). Because *A. ananatis* was originally collected from pineapple growing areas of Brazil and the current culture derived from Kunia (Oahu, HI), a relatively warm site, it might have preadapted to the warm environment and have a high  $T_o$ .

Different species may have different threshold temperatures, and different strains within the same species or even different developmental stages of a species may have different threshold temperatures (Davidson 1944). Bernal and Gonzalez (1993) studied the temperature threshold for parasitoids of the Russian wheat aphid, *Diuraphis noxia* Mordwilko (Homoptera: Aphididae). They found that the  $T_o$  values for the Chile strain of *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) was slightly higher (7.1°C) than the French strain (6.4°C). The low temperature thresholds for development of *Catolaccus grandis* (Burks) eggs, larvae, and pupae were 12, 11.5, and 9.5°C, respectively (Greenberg *et al.* 1996). Similarly, progressively lower thresholds for progressive growth stages of cabbage white butterfly, *Artogeia (Pieries) rapae* L. (Lepidoptera: Pieridae) have been reported (Campbell *et al.* 1974). In *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), a parasitoid of sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), the  $T_o$  value for the immature stages (13.3°C) was determined to be higher than that of the adult (11°C) (Enkegaard 1993). Such a higher threshold for the earlier stages would ensure against possible mortality of later

stages due to cold temperature. The different immature stages of *A. ananatis* may have different  $T_0$  values.

The rate of development of *A. ananatis* increased from 14.6 to 31.0°C, but with a slight deviation from the linearity at both the ends as seen with most insects. The higher threshold temperature could not be determined by simple extrapolation of the linear model, but may be close to 31°C. Continuous exposure to extreme temperatures near the upper or lower thresholds may be deleterious to an organism, but insects can usually sustain exposure to fluctuating high or low temperatures beyond their thresholds for short periods (Tingle and Copland 1988). Under a fluctuating temperature environment, such as in the field, *A. ananatis* might well tolerate occasional exposure to temperatures that may not be considered optimal.

Rearing of three parasitoids of citrus mealybug, *Leptomastix dactylopii*, *Anagyrus pseudococci*, and *Leptomastix abnormis*, at a constant temperature of 40°C was lethal, but all survived a cyclic temperature of 40 and 26°C over 12 hours intervals (Tingle and Copland 1988). The developmental rate of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a biological control agent of purple loosestrife weed, was higher at 30°C than at 25°C. However, its lifetime fecundity was drastically reduced at 30°C compared to 25°C, thereby suggesting that the optimum temperature may be well below the higher threshold temperature (McAvoy and Kok 1999).

For laboratory purposes, the optimal range of temperature for *A. ananatis* was based on the maximal change in the developmental rate and was determined to be 19 - 28.9°C. The optimal temperature fell around 24°C, based on the highest rate of change in the developmental rate (Fig. 6.3). These temperature regimes fall within the range of

previously reported optimal temperatures for the rearing of *Anagyrus kamali* (26°C) and other Anagyrinae (26 - 30°C) (Sagarra *et al.* 2000). Three years (1991 - 1993) of meteorological data were obtained for Kunia, Oahu, a typical pineapple plantation site. The minimum daily temperatures at the site ranged from 11.6 to 22.8°C and maximum daily temperatures ranged from 19.8 to 37.1°C with the mean daily temperatures ranging from 18.2 to 28.4°C. The mean temperatures were always above the  $T_o$  value for *A. ananatis* and within the optimal temperature range. *Anagyrus ananatis* would not stop development within any season due to unfavorable cold temperatures. There was an accumulation of > 4300 DD above the  $T_o$  for *A. ananatis* per year. Although the effect of temperature beyond 31°C was not examined, individuals should be able to handle occasional high temperatures in the pineapple crop microhabitat. If all the heat recorded at the site were accumulated, the parasitoid could complete as many as 16 generations in a year. In contrast, PPM has a relatively long developmental cycle, taking almost two months to reach the mature adult stage at 23°C (Ito 1938). Although the thermal requirements for PPM have not been determined, it could possibly complete 6 to 8 generations in a year within Hawaiian pineapple plantings. The shorter generation time of the *A. ananatis* would be advantageous over the mealybug, providing a tremendous opportunity for a rapid numerical increase.

Developmental stages of *A. ananatis* were similar to *Anagyrus mangicola* Noyes (Cross and Moore 1992). Encyrtiform eggs are characteristic of encyrtids. The eggs and larvae are attached to the host body wall and larval respiration takes place through a stalk. The number of larval instars of encyrtids varies from two to five, with three being more commonly reported (Clausen 1940). Three larval instars were reported for *Anagyrus*



*mangicola*, (Cross and Moore 1992) and four for *Epidinocarsis lopezi* De Santis (Odebiyi and Bokonon-Ganta 1986; Lohr *et al.* 1989). Hypermetamorphosis (where two or more forms of larva are found during a developmental cycle), as observed in *A. ananatis*, is common among parasitic Hymenoptera, with the highest degree of variation in the first instar larvae following the successive molting (Clausen 1940). Well-developed mandibles are used to fight with other competing larvae. Elimination of competitors, found within the same host, by physical attack is common among solitary parasitoids. (Bokonon-Ganta *et al.* 1996). The larvae gradually changed to hymenopteriform, but connection with the host skin through the stalk continued until the larva matured, which is typical of Encyrtiform larva (Clausen 1940). The tremendous increase in body length of the second instar compared to the first instar was also reported for *Anagyrus mangicola* (Cross and Moore 1992). The third instar grows more in width than length and ultimately changed orientation. Reversing the orientation of mature larvae within the host body is common among encyrtids (Clausen 1940; Cross and Moore 1992). Unlike *A. mangicola*, where all individuals orient toward the posterior of the mealybug, *A. ananatis* orients to either end of the mealybug body. The orientation of 82% of larva to the anterior end of mealybug and 85% of the pupa toward the posterior end of the mealybug suggest that *A. ananatis* also changed the orientation during the final larval stage. Larval orientation was dependent on the site of oviposition in the host mealybug. When oviposition occurred in the posterior end of the host body, the larval head was initially directed to the head of the mealybug and finally to the posterior. However, when oviposition occurred in the anterior region then the initial orientation was to the posterior and final head orientation was to the head of the host mealybug. At this stage, the

mealybug body becomes inflated and interior undergoes its typical smoothening (Clausen 1940).

## CONCLUSIONS

*Anagyrus ananatais* has a  $T_0$  around 12.65°C and higher threshold close to 31°C. Few individuals completed their developmental cycle near the lower threshold at 14.6°C. Temperatures from 19 to 29°C are optimal for mass rearing of *A. ananatis*, with the optimal temperature being around 24°C. At this temperature, *A. ananatis* can complete almost two generations in the time it takes its PPM host to complete only one generation. If fluctuating temperature regimes were used to mass produce *A. ananatis*, the development stage of the parasitoid within the mealybug mummy would be useful to estimate the heat accumulated by the parasitoid or *vice versa*. It is also possible to predict the date of adult parasitoid emergence from the initiation of the mummy stage based on the incubation temperature and the development stage. The rearing temperature can be manipulated to shorten or lengthen the developmental time of the parasitoid, but further studies are necessary to evaluate the effect of rearing temperature (within the optimal range) on the parasitoid's biological fitness (reproductive ability and host searching efficiency).

The parasitoid completed its egg to larval development in about eight days, but took much longer time (ca. 14 days) to complete pupal development at 23.5°C. Although the parasitoid is a koinobiont, the host mealybug was killed within a few (6-8) days after parasitization. Further study of effect of parasitization on the biological activities of the host mealybugs (such as progeny production ability) may be useful to better understand

the population dynamics of these insects.

## REFERENCES

- Andrewartha, H. C. and L. C. Birch. 1954. *Distribution and Abundance of Insects*.  
University of Chicago Press, Chicago, 782 pp.
- Bartlett, B. R. 1978. Pseudococcidae, pp. 137-171. *In* *Introduced Parasites and Predators of Arthropod Pest and Weeds: A World Review* (C. P. Clausen Ed.), U. S. Department of Agriculture, Handbook No. 480 Washington D.C.
- Beardsley, J. W. 1993. The pineapple mealybug complex: taxonomy, distribution and host relationships. pp. 383-386. *In* "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First International Pineapple Symposium, 2-6 November, 1992 Honolulu, Hawaii.
- Bernal, J. and D. Gonzalez. 1993. Temperature requirements of four parasites of the russian wheat aphid *Diuraphis noxia*. *Entomologia Experimentalis et Applicata* 69: 173-182.
- Bokonon-Ganta, A. H., J. J. M. V. Alphen and P. Neuenschwander. 1996. Competition between *Gyranusoidea tebygi* and *Anagyrus mangicola*, parasitoids of the mango mealybug, *Rastrococcus invadens*: Interspecific host discrimination and larval competition. *Entomologia Experimentalis et Applicata* 79: 179-185.
- Briere, J. F., P. Pracos, A. Y. L. Roux and J. S. Pierre. 1999. A novel rate model of temperature dependent development for arthropods. *Environmental Entomology* 28: 22-29.

- Campbell, A., B. D. Frazer, N. Gilbert, A. P. Gutierrez and M. Mackaure. 1974.  
 Temperature requirement of some aphid and their parasites. *Journal of Applied Ecology* 11: 431-438.
- Carter, W. 1937. Importation and laboratory breeding of two chalcid parasites of  
*Pseudococcus brevipes* (Ckl.). *Journal of Economic Entomology* 30: 370-372.
- Clausen, C. P. 1940. *Entomophagous Insects*. McGraw Hill Book Co, New York, 688 pp.
- Collins, J. L. 1960. *The Pineapple: Botany, Cultivation and Utilization*. Leonard Hill,  
 London, 295 pp.
- Cross, A. E. and D. Moore. 1992. Developmental studies on *Anagyrus mangicola*  
 (Hymenoptera: Encyrtidae), a parasitoid of the mealybug *Rastrococcus invadens*  
 (Homoptera: Pseudococcidae). *Bulletin of Entomological Research* 82: 307-312.
- Davidson, J. 1944. On the relationship between temperature and rate of development of  
 insects at constant temperatures. *Journal of Animal Ecology* 13: 26-38.
- Enkegaard, A. 1993. *Encarsia formosa* parasitizing the Poinsettia-strain of the cotton  
 whitefly, *Bemisia tabaci*, on Poinsettia: Bionomics in relation to temperature.  
*Entomologia Experimentalis et Applicata* 69: 251-261.
- Funasaki, G. Y., P. Y. Lai, L. M. Nakamura, J. W. Beardsley and A. K. Ota. 1988. A  
 review of biological control introductions in Hawaii 1890-1985. *Proceedings of  
 the Hawaiian Entomological Society* 28: 105-160.
- Gonzalez-Hernandez, H. 1995. *The Status of Biological Control of Pineapple Mealybugs  
 in Hawaii*. Ph.D Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Gonzalez-Hernandez, H., M. W. Johnson and N. J. Reimer. 1999a. Impact of *Pheidole  
 megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of

- Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). Biological Control 15: 145-152.
- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999b. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. BioControl 44: 47-58.
- Greenberg, S. M., J. A. Morales Ramos and E. G. King. 1996. Low temperature effects on development, mortality, fecundity, and viability of the ectoparasitoid *Catolaccus grandis* (Hymenoptera: Pteromalidae). Journal of Entomological Science 31: 391-403.
- Honek, A. 1996. Geographical variation in thermal requirements for insect development. European Journal of Entomology 93: 303-312.
- Ikemoto, T. and K. Takai. 2000. A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. Environmental Entomology 29: 671-682.
- Islam, K. S. and M. Jahan. 1993. Oviposition and development of the mealybug parasitoid *Anagyrus pseudococci* (Girault) at different constant temperatures. Pakistan Journal of Scientific and Industrial Research 36: 322-324.
- Kishore, R., P. Kumar, D. Manjunath and R. K. Datta. 1994. Effect of temperature on the developmental period, progeny production and longevity of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae). Journal of Biological Control 8: 10-13.
- Kotinsky, J. 1910. Coccidae not hitherto recorded from these islands. Proceedings of the Hawaiian Entomological Society 2: 127-131.

- Lide, D. R. 1993. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, 15-20 pp.
- Lohr, B., B. Santos and A. M. Varela. 1989. Larval development and morphometry of *Epidinocarsis lopezi* (DeSantis) (Hym., Encyrtidae), parasitoid of the cassava mealybug, *Phenacoccus manihoti* Mat.-Ferr. (Hom., Pseudococcidae). Journal of Applied Entomology 107: 334-343.
- McAvoy, T. J. and L. T. Kok. 1999. Effects of temperature on eggs, fecundity, and adult longevity of *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), a biological control agent of purple loosestrife. Biological Control 15: 162-167.
- Noyes, J. S. and M. Hayat. 1994. Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae). Natural History Museum/CAB International, London, 554 pp.
- Odebiyi, J. A. and A. H. Bokonon-Ganta. 1986. Biology of *Epidinocarsis* (= *Apoanagyrus*) *lopezi* (Hymenoptera: Encyrtidae), an exotic parasite of Cassava mealybug *Phenacoccus manihoti* (Homoptera: Pseudococcidae) in Nigeria. Entomophaga 31: 251-260.
- Pemberton. 1964. Highlights in the history of entomology in Hawaii 1778-1963. Pacific Insects 6: 689-729.
- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988. Mealybug wilt, mealybugs, and ants of pineapple. Plant Disease 72: 558-565.
- Sagarra, L. A., C. Vincent, N. F. Peters and R. K. Stewart. 2000. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of

- the hibiscus mealybug *Maconellicoccus hirsutus*. *Entomologia Experimentalis et Applicata* 96: 141-147.
- SAS Institute. 1999. The SAS System for Windows release 8.0. SAS Institute, Cary, NC, USA.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by the experiment station of the pineapple producers cooperative association during the period 1930-1938. *Proceedings of the Hawaiian Entomological Society* 10: 352.
- Tingle, C. C. D. and M. J. W. Copland. 1988. Predicting development of the mealybug parasitoids *Anagyrus pseudococci*, *Leptomastix dactylopii* and *Leptomastidae abnormalis* under glasshouse conditions. *Entomologia Experimentalis et Applicata* 46: 19-28.
- Williams, D. J. and G. W. Watson. 1988. The Scale Insects of Tropical South Pacific Region. CAB International, Wallingford, 260 pp.



## VII. STORAGE OF *ANAGYRUS ANANATIS* GAHAN (HYMENOPTERA: ENCYRTIDAE) IMMATURES AT COOL TEMPERATURES

### INTRODUCTION

*Anagyrus ananatais* Gahan (Hymenoptera: Encyrtidae) is the most commonly encountered primary endoparasitoid of pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Homoptera: Pseudococcidae), in Hawaii (Gonzalez-Hernandez *et al.* 1999). It was first imported to Hawaii from Brazil during 1935-1937 (Carter 1937; Sakimura 1939) and has successfully established in the pineapple production areas of Hawaii. The parasitoid has provided partial control of PPM in association with other natural enemies. Field parasitization due to *A. ananatis* in the presence of ants was found to be as high as 9.9% (Funasaki *et al.* 1988; Gonzalez-Hernandez *et al.* 1999). Because of its host specificity, abundance and persistence, *A. ananatis* was chosen as a candidate for an augmentative biological control project targeted against PPM (Gonzalez-Hernandez 1995; Gonzalez-Hernandez *et al.* 1999).

PPM is the most common mealybug infesting pineapple in Hawaii and around the world (Rohrbach *et al.* 1988; German *et al.* 1992; Hughes and Samita 1998; Gonzalez-Hernandez *et al.* 1999). PPM and a closely related species, gray pineapple mealybug (GPM), *Dysmicoccus neobrevipes* Beardsley, are the primary vectors of pineapple mealybug wilt associated virus (PMWaV) (Sether *et al.* 1998). In association with mealybug infestations, PMWaV infections have led to the development of the world's most important pineapple malady, known as mealybug wilt of pineapple, for more than 90 years (Illingworth 1931; Hu *et al.* 1996; Hughes and Samita 1998).

Mass production of a target biological agent is crucial for the implementation of any augmentative biological control program (Morrison and King 1977; van den Bosch *et al.* 1982). The ability to store reared biological control agents provides an opportunity to manufacture them during low demand periods and utilize them during high demand periods. It also permits synchronized field releases of natural enemies during the critical stages of pest outbreaks (Morrison and King 1977; McDonald and Kok 1990; Bueno and van Cleave 1997; Venkatesan *et al.* 2000). The use of cool temperature storage also facilitates the shipping of natural enemies to release sites from production sites as well as permitting timing of releases to accommodate work schedules and periods of inclement weather (Whitaker-Deerberg *et al.* 1994; Bueno and van Cleave 1997). It also helps to maintain parasitoids when not needed and to minimize laboratory operations by prolonging parasitoid survival and delaying eclosion to the adult stage (Bueno and van Cleave 1997).

Cool temperatures used to slow down developmental rates are the primary means utilized in the storage of most parasitoids (Morrison and King 1977). Several factors (e.g., survival, sex ratio, reproductive ability and behavior) have to be considered before implementing cool storage of entomophages for practical purposes (Etzel and Legner 1999). Attempts to store insects at various stages (e.g., egg, prepupa, pupa and adult) have been made. Common green lacewing eggs stored at 13-14°C for more than ten days had reduced viability (Morrison 1985), but eggs of the whiteflies *Bemisia tabaci* Gennadius and *B. argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) stored at 10°C for 28 days had 56% survival (Lacey *et al.* 1999). Prepupae of *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) survived storage at 10°C and produced viable

progeny up to 14 months afterward (McDonald and Kok 1990). Pupae of *Sturmiopsis inferens* Townsend (Diptera: Tachinidae), a larval parasitoid of sugarcane moth borer were stored for 30 days at 15°C without any adverse effects on fly emergence, female mating or male longevity (Easwaramoorthy *et al.* 2000). Krishnamoorthy (1989) reported better survival of *Leptomastix dactylopii* Howard immatures when stored as mummies rather than adults at 15°C. Survival has been the most important indicator of fitness of stored insects, but reproductive ability has also received some attention.

Earlier studies indicated that *A. ananatis* has a lower developmental threshold ( $T_o$ ) of 12.65°C and that females and males require approximately 275 and 265 degree days (DD), respectively, to complete development from egg to adult (see Chapter 6). A preliminary study showed that *A. ananatis* prepupal and pupal stages could be stored for over six weeks at 15°C without affecting their eclosion rate, but storage at 10°C killed most of the individuals and eclosion was poor. Also, storing the parasitoids at the pink eye stage allowed them to tolerate cool temperatures better, but the safe storage period was shortened to three weeks.

The studies reported here were conducted to investigate the effect of storage of *A. ananatis* prepupal and pupal stages at temperatures above and below its  $T_o$ , with and without exposing them to a pre-conditioning temperature treatment. The effects of cool temperature storage on various adult fitness parameters (i.e., longevity, fecundity) were evaluated for adults that underwent cool storage as immatures.

## MATERIALS AND METHODS

PPM and *A. ananatis* individuals used in this study were obtained from a culture maintained at the Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu. PPM were reared as described in Chapter 3.

### **Survival of Cool Storage and adult longevity**

About 50 female and 25 male newly eclosed, adult wasps were transferred to screen cages (30 cm x 14 cm x 12 cm) and provided with honey on a glass slide and water through a cotton wick. Wasps were held in a temperature cabinet at  $25 \pm 1^\circ\text{C}$ ,  $55 \pm 5\%$  RH, and 14:10 (L: D) hours and denied access to PPM. After three days of host deprivation, about 400 mature adult mealybugs were equally divided into four cups and placed into each cage. After 24 hours, the parasitized mealybugs were removed from the oviposition cage and held in another cage in the same cabinet until used in experiments.

Following the accumulation of approximately 100 to 120 DD (around 9-10 days following oviposition at  $25^\circ\text{C}$ ), *A. ananatis* reaches the prepupal stage and their mealybug host's integument changes shape and hardens into the form referred to as a mummy (see Chapter 6). At the same temperature, the prepupal stage molts into the pupal stage in 11-12 days (after accumulating 120 to 145 DD of heat). Dark meconial pellets are excreted by parasitoid individuals at one end of the oval-shaped mummy prior to pupation. On Day 12 following parasitization (around 140 DD), when most of the larvae were pupating within the mummies, they were placed in glass tubes (10 ml, Fisherbrand disposable culture tubes) in batches of 25 mummies per tube. The tubes were wrapped with aluminum foil (to eliminate light) and plugged with a dental cotton roll section (38 x 10 mm) (Henry Schein, Middlesex, England). The tubes with the *A.*

*ananatis* immatures were stored in a container (40 cm x 55 cm x 33 cm) with a snap tight lid (Clear Stack on Wheels™, IRIS USA INC, Pleasant Prairie, WI). The humidity was maintained at 75% RH with a saturated salt (NaCl) solution (Lide 1993). Temperature and relative humidity of the chamber were continuously monitored with a data logger (Dickson TH Trace TL120) every 15 minutes throughout the experimental period.

In this study, storage period and temperature were both varied. Storage period treatments included 2, 4, 6, and 8 weeks cool storage under varying conditions compared to no storage for *A. ananatis* individuals reared at  $25 \pm 1^\circ\text{C}$ . Temperatures used for storage periods included  $14.8^\circ\text{C}$  (above the  $T_o$ ),  $10.1^\circ\text{C}$  (below the  $T_o$ ), and a combination of preconditioning for one week at  $14.8^\circ\text{C}$  followed by 1, 3, 5 and 7 weeks at  $10.1^\circ\text{C}$ . These treatments were replicated four times each.

At the end of the storage period, the *A. ananatis* immatures within mummies were removed from cool storage and transferred individually to clear gelatin capsules (size 2) and held at  $25 \pm 1^\circ\text{C}$ ,  $55 \pm 5\%$  RH and 14:10 (L: D) hours photoperiod to complete eclosion. Time taken (days) to emerge from mummies after transfer to  $25^\circ\text{C}$ , total emergence and sex of the emerged individuals were noted. Percentage emergence was calculated from the number of mummies from which adult parasitoids successfully emerged. The mummies from which parasitoids failed to emerge were dissected and the stage (prepupa, pupa, adult) of wasp inside was recorded. These numbers were also converted into percentages.

Five female parasitoids from each treatment combination were transferred to clear polystyrene tubes (20 cm long x 9 cm diameter), provided with honey and water and observed daily for survival until they all died.

## **Progeny Production**

Based on the results of emergence and longevity, the following treatments were selected for progeny production evaluation: preconditioning at 14.8°C for one week and storage at 10.1°C for one week; storage at 14.8°C for 2, 4, 6, and 8 weeks; and an untreated control maintained at  $25 \pm 1^\circ\text{C}$ . Five female parasitoids, each having undergone the above temperature treatments, were used to determine the effect of storage on their ability to produce offspring. Each female was paired with one male wasp from the same treatment and transferred to a tube cage and provided with honey and water. Each pair was provided with 25 adult PPM per day for five consecutive days after emergence. The numbers of mummified mealybugs resulting from PPM exposure to test females were counted 14 days after exposure. Individual mummies were placed in a gelatin capsule and held at  $25 \pm 1^\circ\text{C}$  until emergence and the sex of the emerging parasitoid was determined.

*Statistical Analysis.* The time required for adult emergence following removal of parasitoids from cool storage was analyzed using ANOVA procedures, Proc GLM, (SAS Institute 1999). Storage temperature, storage period and parasitoid sex and their interactions (standard three level interactions) were the independent variables examined. Percentage emergence and the percentages of mummies that failed to emerge, but developed to different life stages (prepupa, pupa and adult) were Arcsine square root transformed and analyzed with ANOVA procedures with storage temperature, storage period and their interactions as independent variables. The longevity of individual wasps was  $\text{Log}_{10}(x)$  transformed and analyzed with ANOVA procedures with storage temperature, storage period and their interactions as independent variables.

Progeny production was evaluated by estimating the mean daily number of mummies resulting from oviposition of individual female parasitoids over a five-day period. If a female parasitoid reproduced, then it was considered a functional individual. When the progeny included female parasitoids, the male, which also underwent cool storage, was considered functional. Number of progeny produced per female was log transformed and analyzed with ANOVA procedures.

## RESULTS

### **Survival of Cool Storage**

*Emergence rate.* The mean emergence rate in the untreated check was 95.1% (Table 7.1). There was a statistically significant interaction between the storage temperature and length of storage period ( $F_{6, 36} = 15.8, P = 0.0001$ ). Likewise, the effects of storage temperature ( $F_{2, 36} = 423.6, P < 0.0001$ ) and storage period ( $F_{3, 36} = 38.6, P < 0.0001$ ) were significant. Emergence rates for individuals stored at 14.8°C (above  $T_o$ ) ranged from about 90 to 98%, but were not significantly different and were comparable to that in the control. The immatures preconditioned at 14.8°C for one week and then stored at 10.1°C for one week had significantly higher (83%) emergence than those stored for 4 to 6 weeks (56-52 %). Even significantly fewer individuals emerged (21%) when stored for 8 weeks. Parasitoid emergence was severely affected when they were stored at 10.1°C (below the  $T_o$ ) without preconditioning. The emergence rate dropped to 46% for individuals stored two weeks and to less than 3% after six weeks storage. No individuals emerged after 8 weeks storage.

Table 7. 1. Percent emergence of *Anagyrus ananatis* adults following cool temperature storage under various conditions during immature stages.

Storage period (weeks)	Storage temperature (°C)			
	10.1 <sup>a,e</sup>	14.8 / 10.1 <sup>b,e</sup>	14.8 <sup>c,e</sup>	25 <sup>d,e</sup>
0	—	—	—	95.1 ± 1.8
2	46.3 ± 9.1 a	83.0 ± 2.6 a	90.2 ± 2.4 a	—
4	7.0 ± 1.9 b	56.0 ± 4.5 b	94.2 ± 1.9 a	—
6	2.9 ± 0.9 b	52.0 ± 6.0 b	98.0 ± 1.1 a	—
8	0.0 ± 0.0 b	21.2 ± 2.7 c	93.2 ± 1.8 a	—

<sup>a</sup> Below lower developmental threshold ( $T_o$ )

<sup>b</sup> Preconditioning for one week above the  $T_o$  (14.8°C) followed by storage below the  $T_o$  (10.1°C)

<sup>c</sup> Above  $T_o$

<sup>d</sup> No cool storage (check treatment)

<sup>e</sup> Data arcsine square root transformed before analysis. The groups followed by the same letters are not significantly different from each other within a column ( $P = 0.05$ ).



## Identification of Stages Not Surviving Cool Storage

Mummies from which viable adults did not emerge were dissected and the various phenological stages of individuals within identified.

*Prepupal stage.* In terms of parasitoids dying in the prepupal stage while in cool storage, significant differences were found in regards to the effects of storage temperature ( $F_{2,36} = 168.2, P < 0.0001$ ), storage period ( $F_{3,36} = 5.6, P = 0.0030$ ), and the interaction between the two variables ( $F_{6,36} = 5.0, P = 0.0008$ ) (Table 7.2). No individuals within the untreated control, the 14.8°C regime or the 14.8°C/10.1°C preconditioning regime died in the prepupal stage except in the eight week storage treatment in the preconditioned regime. All individuals in these treatments, except for the treatment denoted, developed to the pupal or adult stages. Storage at 10.1°C without preconditioning was detrimental to parasitoid development. When immatures were stored for two weeks at 10.1°C, about 7% of the individuals died as prepupae, which was significantly less than the percent dead prepupae (23 to 28%) found among individuals stored greater than 2 weeks at 10.1°C (Table 7.2).

*Pupal stage.* In terms of parasitoids dying in the pupal stage while in cool storage, significant differences were found regarding the effects of storage temperature ( $F_{2,36} = 408.3, P < 0.0001$ ), storage period ( $F_{3,36} = 24.6, P = 0.0001$ ) and the interaction between the two variables ( $F_{6,36} = 11.7, P = 0.0001$ ) (Table 7.2). Few individuals died in the pupal stage when they were stored at 14.8°C. The proportion of pupae that failed to develop to the adult stage gradually and significantly increased with an increase in the storage period for both treatment groups stored at 10.1°C. However, when individuals were preconditioned at 14.8°C prior to storage at 10.1°C, only 12% percent died as pupae

Table 7. 2. Mean percentages of dead *Anagyrus ananatis* individuals that were found in the prepupal, pupal, and adult stages within PPM mummies following cool temperature storage of immature stages.

Storage period (weeks)	Storage temperature			
	10.1 <sup>a,e</sup>	14.8 / 10.1 <sup>b,e</sup>	14.8 <sup>c,e</sup>	25.0 <sup>d,e</sup>
Prepupal stage				
0	—	—	—	0.0 ± 0.0
2	6.8 ± 3.4 a	0.0 ± 0.0 a	0.0 ± 0.0 a	—
4	24.0 ± 5.6 b	0.0 ± 0.0 a	0.0 ± 0.0 a	—
6	27.9 ± 5.5 b	0.0 ± 0.0 a	0.0 ± 0.0 a	—
8	22.6 ± 2.0 b	1.0 ± 1.0 a	0.0 ± 0.0 a	—
Pupal stage				
0	—	—	—	1.0 ± 1.0
2	28.8 ± 5.6 a	11.9 ± 1.6 a	2.0 ± 1.1 a	—
4	53.0 ± 3.4 b	30.7 ± 6.8 b	0.0 ± 0.0 a	—
6	68.2 ± 5.7 bc	39.2 ± 3.1 b	0.0 ± 0.0 a	—
8	75.5 ± 2.9 c	48.4 ± 4.7 b	0.0 ± 0.0 a	—
Adult stage				
0	—	—	—	3.9 ± 2.3
2	18.1 ± 4.1 a	5.1 ± 2.0 a	7.8 ± 2.1 a	—
4	16.0 ± 5.9 a	13.2 ± 2.4 a	5.7 ± 1.9 a	—
6	1.0 ± 1.0 b	8.7 ± 3.4 a	2.0 ± 1.1 a	—
8	1.9 ± 1.9 b	29.4 ± 7.3 b	6.7 ± 1.8 a	—

<sup>a</sup> Below lower developmental threshold ( $T_o$ )

<sup>b</sup> Preconditioning for one week above the  $T_o$  (14.8°C) followed by storage below the  $T_o$  (10.1°C)

<sup>c</sup> Above  $T_o$

<sup>d</sup> No cool storage (check treatment)

<sup>e</sup> Data arcsine square root transformed before analysis. The groups followed by the same letters are not significantly different from each other within a column ( $P = 0.05$ )

during two weeks of storage compared to 28.8 percent without preconditioning at 14.8°C. These mortality values increased to 30 to 48% during 4 to 8 weeks of storage. Without preconditioning, pupal mortality ranged from 53 to 75% during 4 to 8 weeks of storage.

*Adult stage.* The storage period and storage temperature interacted significantly in terms of the number of individuals that developed to the adult stage, but failed to emerge ( $F_{6, 36} = 5.7, P = 0.0003$ ) (Table 7.2). When individuals were stored at 14.8°C, 2 to 8% developed to the adult stage, but died before emerging from the mummy. No significant differences were found in *A. ananatis* adult mortality within the mummies among storage times within the 14.8°C regime, but differences were evident in individuals stored at both 10.1°C regimes ( $F_{2, 36} = 4.7, P < 0.0151$ ).

When stored for six weeks or less, 5.1 to 13.2% of preconditioned individuals failed to emerge from the mummies after reaching the adult stage, but adult mortality significantly increased to 29.4% when individuals were stored for 8 weeks ( $F_{3,36} = 4.8, P = 0.0064$ ). When the individuals were stored at 10.1°C without preconditioning, adult mortality within the mummies ranged from 18 to 16% during 2 to 4 weeks storage, but significantly decreased to less than 2% for individuals stored for 6 to 8 weeks. This is somewhat misleading because higher percentages of pupal death occurred in the 6 and 8 week storage periods at 10.1°C, thereby reducing the numbers of individuals surviving to the adult stage.

### **Effects on Developmental Time and Adult Longevity**

*Days to emergence after storage.* The time to emergence for the immatures stored at 14.8°C varied significantly from the other temperature regimes ( $F_{2,61} = 1175.3, P < 0.0001$ ) and gradually decreased as the length of the storage period increased ( $F_{3,61} =$

227.0,  $P < 0.0001$ ) (Table 7.3). Individuals stored at 10.1°C had an overall developmental period (when at 25°C) comparable to that of the control group. The storage temperature of 14.8°C was effectively higher than the  $T_o$  and 10.1°C was effectively lower than the  $T_o$ , which also supported the previously determined  $T_o$  of 12.65°C (see Chapter 6). The estimated DD accumulated by individuals, while stored under the various temperature regimes prior to their return to 25°C, are provided (Table 7.4).

There was no significant three-way interaction among storage temperature, storage period and sex in terms of the time taken to for adult emergence from the mummy after individuals were returned to 25°C ( $F_{4,61} = 0.29$ ,  $P = 0.8848$ ) (Table 7.3). Likewise, there were no significant interactions between sex of the parasitoid and storage temperature ( $F_{2,61} = 0.57$ ,  $P = 0.5694$ ) or storage period ( $F_{3,61} = 0.36$ ,  $P = 0.7847$ ). The male wasps emerged significantly earlier than the females irrespective of the treatments applied ( $F_{1,61} = 9.43$ ,  $P = 0.0032$ ).

There was a significant interaction between storage temperature and the storage period ( $F_{5,61} = 162.5$ ,  $P < 0.0001$ ). Females and males in the untreated check emerged in 11.6 and 11.3 days, respectively, after their cohorts were stored. For females stored at 14.8°C (above the  $T_o$ ), time to emergence, following return to 25°C, gradually decreased from 9.6 to 1.3 days from 2 to 8 weeks cool storage, respectively. For males, the time decreased from 9.2 to 1.0 days from 2 to 8 weeks storage, respectively. No *A. ananatis* females stored at 10.1°C (below the  $T_o$ ) for 8 weeks without preconditioning completed development to the adult stage. The same result was recorded for males stored for 6 and 8 weeks. Under the same temperature regime, females stored from 2 and 4 weeks

Table 7. 3. Mean time periods (days) required by *Anagyrus ananatis* immatures, following cool storage under various conditions, to eclose following incubation at  $25 \pm 1^\circ\text{C}$ .

Storage period (weeks)	Storage Temperature ( $^\circ\text{C}$ )			
	10.1 <sup>a,e</sup>	14.8 / 10.1 <sup>b,e</sup>	14.8 <sup>c,e</sup>	25 <sup>d,e</sup>
Female				
0	—	—	—	11.6 $\pm$ 0.04
2	12.1 $\pm$ 0.22 a	10.4 $\pm$ 0.07 a	9.6 $\pm$ 0.09 a	—
4	12.0 $\pm$ 0.00 a	9.1 $\pm$ 0.37 b	6.9 $\pm$ 0.25 b	—
6	14.3 $\pm$ 0.67 b	9.2 $\pm$ 0.24 b	4.1 $\pm$ 0.07 c	—
8	—	9.4 $\pm$ 0.23 b	1.3 $\pm$ 0.25 d	—
Male				
0	—	—	—	11.3 $\pm$ 0.11
2	11.8 $\pm$ 0.28 a	10.2 $\pm$ 0.05 a	9.2 $\pm$ 0.15 a	—
4	11.7 $\pm$ 0.25 a	9.1 $\pm$ 0.19 ab	6.3 $\pm$ 0.16 b	—
6	—	8.8 $\pm$ 0.12 b	3.5 $\pm$ 0.13 c	—
8	—	9.1 $\pm$ 0.24 ab	1.0 $\pm$ 0.34 d	—

<sup>a</sup> Below lower developmental threshold ( $T_o$ )

<sup>b</sup> Preconditioning for one week above the  $T_o$  ( $14.8^\circ\text{C}$ ) followed by storage below the  $T_o$  ( $10.1^\circ\text{C}$ )

<sup>c</sup> Above  $T_o$

<sup>d</sup> No cool storage (check treatment)

<sup>e</sup> The groups followed by the same letters are not significantly different from each other within a column for each sex ( $P = 0.05$ ).

Table 7. 4. Estimated Degree-Days accumulated by *Anagyrus ananatis* immatures while held in cool storage under various conditions.

Storage period (weeks)	Storage Temperature (°C)			
	10.1 <sup>a</sup>	14.8 / 10.1 <sup>b</sup>	14.8 <sup>c</sup>	25 <sup>d</sup>
0	—	—	—	NA
2	0	15.05	30.1	—
4	0	15.05	60.2	—
6	0	15.05	90.3	—
8	0	15.05	120.4	—

<sup>a</sup> Below lower developmental threshold ( $T_0$ )

<sup>b</sup> Preconditioning for one week above the  $T_0$  (14.8°C) followed by storage below the  $T_0$  (10.1°C)

<sup>c</sup> Above  $T_0$

<sup>d</sup> No cool storage (check treatment)

NA = Not applicable

completed development in about 12 days which was significantly shorter than the time (14.3 days) required for females stored for 6 weeks. Males stored for 2 and 4 weeks at 10.1°C completed development in less than 12 days after being returned to 25°C. Individuals preconditioned at 14.8°C for one week followed by storage at 10.1°C had shorter developmental periods (females: 9.1 to 10.4 days; males: 8.8 to 10.2 days) following the return to 25°C than the control as well as those stored at 10.1 °C without preconditioning.

*Adult longevity.* Storage temperature did not significantly affect adult female longevity ( $F_{2, 156} = 1.62, P = 0.2004$ ), but storage period did have a highly significant effect ( $F_{3, 156} = 5.62, P = 0.0011$ ) (Table 7.5). Mean longevity for untreated females was about 32 days. When female immatures were stored at 14.8°C, their mean longevity ranged from 12.9 to 22.8 days, but no significant differences were detected. Because some of the individuals stored for 8 weeks had emerged from the PPM mummies before they were returned to 25°C, the sub-sample size was decreased to 15 instead of 20. Because emergence was poor in the 10.1°C treatment, only 4-7 wasps were used to measure adult longevity of individuals stored for 2 and 4 weeks at temperatures below 10.1°C without preconditioning. Individuals stored for 2 and 4 weeks lived as adults for means of 33 and 34 days, respectively. Inadequate numbers of wasps emerged after 6 and 8 weeks storage at 10.1°C. Thus, adult longevity was not measured for those treatments. Females emerging from preconditioned immatures lived for 26 to 31 days when the storage periods were 2 to 6 weeks. Only 7 individuals emerged from storage for 8 weeks. Their adult longevity was significantly shorter (8.2 days) than individuals stored for shorter periods.

Table 7. 5. Mean longevity (days) of *Anagyrus ananatis* adult females following cool storage during immature stages.

Storage period (weeks)	Storage temperature (°C)			
	10.1 <sup>a,e</sup>	14.8 / 10.1 <sup>b,e</sup>	14.8 <sup>c,e</sup>	25.0 <sup>d,e</sup>
0	—	—	—	31.8 ± 3.9
2	33.0 <sup>f</sup> ± 5.5 a	31.3 ± 3.3 a	22.8 ± 4.4 a	
4	34.0 <sup>f</sup> ± 7.0 a	26.3 ± 3.8 a	21.2 ± 2.4 a	
6	—	30.1 ± 3.3 a	14.7 ± 2.8 a	
8	—	8.2 <sup>f</sup> ± 4.6 b	12.9 <sup>f</sup> ± 2.6 a	

<sup>a</sup> Below lower developmental threshold ( $T_0$ )

<sup>b</sup> Preconditioning for one week above the  $T_0$  (14.8°C) followed by storage below the  $T_0$  (10.1°C)

<sup>c</sup> Above  $T_0$

<sup>d</sup> No cool storage (check treatment)

<sup>e</sup> Data  $\text{Log}_{10}$  transformed before analysis. The groups followed by the same letters are not significantly different from each other within a column ( $P = 0.05$ ).

<sup>f</sup> All treatments replicated 20 times except for the following where replications (n) are indicated in parentheses: 10.1 °C at 2 weeks (7) and 4 weeks (4); 14.8 / 10.1 °C at 8 weeks (7); 14.8 °C at 8 weeks (15).



## Progeny Production

There were significant differences among the females from the various treatments in regards to mean daily production of female ( $F_{5,24} = 16.21, P < 0.0001$ ), male ( $F_{5,24} = 7.27, P = 0.0003$ ), and total progeny ( $F_{5,24} = 18.39, P < 0.0001$ ) (Table 7.6). All the five tested females in the control and two week storage groups produced both male and female progeny. Two females from the four week storage group and only one female from the eight-week storage produced progeny, which were all males. Females that survived the six week storage did not produce any progeny. The control parasitoids produced an average of 6 progeny per female per day. The adults held as immatures at 14.8°C and then 10.1°C (2 weeks total), produced 4 progeny per female per day, which was not significantly different from the control females.

Storage of immatures at 14.8°C for two weeks resulted in the production of only 1.8 female progeny per female per day, which was almost an 80% reduction in female progeny production compared to the control value.

## DISCUSSION

*Anagyrus ananatis* individuals stored above the  $T_o$  (14.8°C) had emergence rates comparable to the control indicating high survival rates at that temperature. The temperature 10.1 °C (below the  $T_o$ ) was deleterious to parasitoid survival, especially when individuals were stored without preconditioning. Preconditioning by exposing the individuals to a cool temperature (14.8°C) above, but close to, the  $T_o$  improved parasitoid survival. Effects were severe when *A. ananatis* individuals were stored at 10.1°C while in

Table 7. 6. Mean number of progeny produced per day by *Anagyrus ananatis* females that previously underwent cool storage under various temperature and storage conditions during their immature stages.

Storage temperature (°C)	Storage period (weeks)	No. fecund females <sup>a</sup>	No. of progeny		
			Male	Female	Total
25.0 <sup>b</sup>	0	5	2.16 ± 0.55 a	3.84 ± 0.58 a	6.00 ± 1.07 a
14.8 / 10.1 <sup>c</sup>	2	5	1.84 ± 0.37 ab	2.24 ± 0.70 ab	4.08 ± 0.73 ab
14.8 <sup>d</sup>	2	5	1.08 ± 0.43 abc	0.76 ± 0.32 bc	1.84 ± 0.50 bc
	4	2	0.36 ± 0.27 c	0.00 ± 0.00 c	0.36 ± 0.27 c
	6	0	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
	8	1	0.08 ± 0.08 c	0.00 ± 0.00 c	0.08 ± 0.08 c

<sup>a</sup> No. of females that were fecund out of group of five females in test

<sup>b</sup> No cool storage (check treatment)

<sup>c</sup> Preconditioning for one week above the lower developmental threshold (T<sub>o</sub>) (14.8°C) followed by storage below the below T<sub>o</sub> (10.1°C)

<sup>d</sup> Above T<sub>o</sub>

the prepupal stage because most failed to develop further. Individuals that were preconditioned at 14.8°C before being held at 10.1°C experienced no mortality in the prepupal stage. The one week of storage at 14.8°C allowed all prepupae to develop into the pupal stage before they were transferred to 10.1°C.

The  $T_o$  decreases in many insect species as they advance to later growth stages. This change enables them to better tolerate cool temperatures. Older immatures have been reported to have better cold tolerance than younger immatures in *Aphelinus perpallidus* (Gahan) (Hymenoptera: Aphelinidae), a parasitoid of black margined aphid of pecan (Bueno and van Cleave 1997). Conditioning of *A. perpallidus* immatures either before or after cold storage had a positive impact on parasitoid adult emergence. Similarly, immatures of *Gonizus nephantidis* Muesebeck (Hymenoptera: Bethylinidae), a parasitoid of black headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae), attacking coconut had higher survival in cool temperatures when subjected to cool temperature storage 48 hours after spinning their cocoons rather than at 24-hours post-cocoon construction (Venkatesan *et al.* 2000). There was no emergence after 20 days of storage of immatures at 5°C whereas there was 100% emergence when stored at 15°C.

When the storage temperature (i.e., 14.8°C) for *A. ananatis* was higher than the  $T_o$  (12.65°C), the post-storage period at 25°C gradually decreased as the storage period increased. For each two weeks of storage at 14.8°C, the developmental period to the adult stage decreased by 2.0 and 2.9 days for males and females, respectively. Based on the  $T_o$ , the parasitoids would accumulate about 2.15 DD per day and a total of 30.1 DD in two weeks (Table 7.4). If the parasitoids were held at 25°C, they would accumulate

12.35 DD each day and require between 2 to 3 days to accumulate the same amount of heat. The parasitoids, which were stored at 10.1°C without preconditioning, required a similar amount of time to emerge from the mummies as the control group, except for the one cohort stored for six weeks in which the time was significantly higher than those in storage for 4 weeks or less. This significantly prolonged time required to emerge could be due to some effect of the cool temperature on insect physiology. Similar results were found with *Aphelinus aschysis* Walker (Hymenoptera: Aphelinidae) in which the total incubation period for individuals stored at 4.4°C slightly increased with an increase in the storage period compared to the control (Whitaker-Deerberg *et al.* 1994). It could be because of a retarded growth rate or selective survival of individuals that have a slower growth rate. Retarded growth rates, owing to exposure to extreme temperatures, have long been identified and are dependent on the duration of exposure (Andrewartha and Birch 1954). On the other hand, because only a few (< 3%) individuals survived in this treatment, only a few that had longer developmental time might have been selected.

The females produced from the immatures preconditioned for one week above  $T_0$  followed by storage for one week below  $T_0$  had no impact on their reproductive ability compared to the control. However, storage of immatures continuously for two weeks or more above  $T_0$  had a deleterious effect on the reproductive ability of *A. ananatis*. Although cool temperature storage affected progeny production by females, males were probably more severely affected. Females stored as immatures for four weeks or more did not produce any female progeny, suggesting a lack of fertilization by stored males. This could be due to several reasons, most apparent being to the inability of the males to mate or their failure to produce viable sperm. In *Brachymeria intermedia* (Nees)

(Hymenoptera: Chalcididae), fewer males survived prolonged cold storage than females. (Palmer 1985). Clausen (1978) reported that prolonged exposure of adult *Aphidius smithii* Sharma and Subbarao inactivated the sperm in males as well as in mated females.

Development of insect organs may be affected differently at different temperatures. The longer the storage period above the  $T_o$ , the longer was the time that developing organs were exposed to sub-optimal temperatures. Although *A. ananatis* survived prolonged exposure to a low temperature near the  $T_o$ , development of the reproductive organs and the endocrine system at a lower than normal temperature may have affected their normal functioning. Andrewartha and Birch (1954) pointed out that animals exposed to extreme low or high temperatures outside their limits of favorable temperature range may continue to survive for long periods, but fail to develop or reproduce. Sterility in the adult stage, as a result of exposure to unfavorably high temperatures in the prepupal and pupal stages, has been reported by Andrewartha and Birch (1954). Similar effects of cool temperature also may exist. Many previous cool storage studies have indicated reduced fecundity following cool temperature storage. Puparia of *Sturmiopsis inferens* Townsend (Diptera: Tachnidae) stored at 15°C exhibited a slight reduction in adult fecundity (Easwaramoorthy *et al.* 2000). Cool storage of *Allorhogas pyralophagus* Marsh (Hymenoptera: Braconidae) immatures within their cocoons had a deleterious effect on fecundity (Ballal *et al.* 1989). *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) longevity and fecundity were adversely affected following cool temperature storage (Ganteaume *et al.* 1995).

Results herein suggest that prolonged exposure to a temperature below the  $T_o$  killed *A. ananatis* prepupae, but the pupae were tolerant to some extent. Exposure to

extreme temperatures may kill an animal instantly, but moderate cool temperatures require considerable time before killing an insect. This is known as the 'quantity factor' (Andrewartha and Birch 1954). The high subsequent death rate of parasitoids before completing their lifecycle may be attributed to depletion of energy reserves during cool storage (Bueno and van Cleave 1997). The effects of short exposure to extremely high temperatures followed by a return to a favorable temperature indicate that an organism may develop and grow apparently normally until some critical stage is reached (Andrewartha and Birch 1954). Similar effects of exposure to cold temperature may occur, allowing normal development for sometime, but causing death before reaching the next stage of development.

## CONCLUSIONS

*Anagyrus ananatis* has a wide range of variability in cold temperature tolerance. Previous studies have shown that they developed well at temperature ranges from 19 to 31°C (see Chapter 6). The effects of cool temperature storage were time dependent, with longer storage periods inducing higher mortality and lowered fecundity. Current results also showed that immatures within mummies could be stored up to 6 weeks at 14.8°C without affecting their survival and longevity, but their fecundity would be severely affected.

Preconditioning of immatures at a cold temperature (above the  $T_0$ ) for one week before exposing them to a temperature below the  $T_0$  reduced the deleterious effect of cold temperature and improved survival rate and fecundity compared to those without preconditioning. Preconditioning of the immatures at 14.8°C for one week and then

storage at 10.1°C resulted in relatively higher emergence as well as higher fecundity compared to longer storage periods and may be employed for storage as well as transportation of the mummies. Prepupal to early pupal stages can only be stored for brief periods (< 2 weeks) at 10.1°C (below the  $T_o$ ), because prolonged exposure is lethal.

Alternatively, manipulation of rearing temperatures within the optimum development range (19-29°C) may be appropriate to delay or hasten the mass production of the parasitoid (see Chapter 6). Also, adult parasitoids lived for about a month at  $24 \pm 1^\circ\text{C}$  when they were provided with honey and water (see Chapter 4). Possibilities of storing adults should be evaluated because a closely related species, *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), was successfully stored for > 40 days at 20°C (Sagarra *et al.* 2000).

Many studies have used survival, longevity and sex ratio as the major parameters to evaluate the effects of cool temperature storage. Although these traits are important, the most important traits are reproductive ability coupled with host searching ability. These results suggest that survival and longevity may give faulty estimates of the fitness of the stored parasitoid. The effect of cold temperature appears to be more detrimental to male parasitoids than females. Further studies are necessary to confirm this preliminary finding.

## REFERENCES

- Andrewartha, H. C. and L. C. Birch. 1954. Distribution and Abundance of Insects.  
University of Chicago Press, Chicago, 782 pp.
- Ballal, C. R., S. K. Jalali and P. Kumar. 1989. Cold storage of Coccons of *Allorhogas pyralophagus* (Hymenoptera: Braconidae). *Entomophaga* 34: 463-468.
- Bueno, R., Jr. and H. S. van Cleave. 1997. The effect of cold storage on the emergence of *Aphelinus perpallidus*, a parasitoid of *Monellia caryella*. *Southwestern Entomologist* 22: 39-51.
- Carter, W. 1937. Importation and laboratory breeding of two chalcid parasites of *Pseudococcus brevipes* (Ckl.). *Journal of Economic Entomology* 30: 370-372.
- Clausen, C. P. 1978. Hemiptera: Aphididae, pp. *In* Introduced Parasites and Predators of Arthropod Pest and Weeds: A World Review (C. P. Clausen Ed.), U. S. Department of Agriculture, Handbook No. 480, Washington D.C.
- Easwaramoorthy, S., N. K. Kurup, G. Santhalakshmi and M. Shanmugasundaram. 2000. Effect of low temperature storage on the viability of puparia of *Sturmiopsis inferens* Townsend (Diptera: Tachnidae) a larval parasitoid of sugarcane moth borers. *Journal of Biological Control* 14: 63-65.
- Etzel, L. K. and E. F. Legner. 1999. Culture and colonization, pp. 125-198. *In* Handbook of Biological Control: Principles and Applications of Biological Control (T. S. Bellows, Jr. and T. W. Fisher Ed.), Academic Press, San Diego.



- Funasaki, G. Y., P. Y. Lai, L. M. Nakamura, J. W. Beardsley and A. K. Ota. 1988. A review of biological control introductions in Hawaii 1890-1985. *Proceedings of the Hawaiian Entomological Society* 28: 105-160.
- Ganteaume, A., E. Tabone and N. Poinso-Balaguer. 1995. Variation in biotic potentialities of *Encarsia formosa* after black pupae cold storage. 2. Variation in daily fecundity and longevity. *Journal of Applied Entomology* 119: 547-551.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. *Advances in Disease Vector Research* 9: 241-259.
- Gonzalez-Hernandez, H. 1995. The Status of Biological Control of Pineapple Mealybugs in Hawaii. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- Hu, J. S., D. M. Sether and D. E. Ullman. 1996. Detection of pineapple Closterovirus in pineapple plants and mealybugs using monoclonal antibodies. *Plant Pathology* 45: 829-836.
- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.
- Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Krishnamoorthy, A. 1989. Effect of cold storage on the emergence and survival of the adult exotic parasitoid, *Leptomastix dactylopii* How. (Hymenoptera, Encyrtidae). *Entomon* 14: 313-318.

- Lacey, L. A., L. Millar, A. A. Kirk and T. M. Perring. 1999. Effect of storage temperature and duration on survival of eggs and nymphs of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and pupae of the whitefly parasitoid *Encarsia formosa* (Hymenoptera: Aphelinidae). *Annals of the Entomological Society of America* 92: 430-434.
- Lide, D. R. 1993. *CRC Handbook of Chemistry and Physics*. CRC Press, Boca Raton, 15-20 pp.
- McDonald, R. C. and L. T. Kok. 1990. Post refrigeration viability of *Pteromalus puparum* (Hymenoptera: Pteromalidae) prepupae within host chrysalids. *Journal of Entomological Science* 25: 409-413.
- Morrison, R. K. and E. G. King. 1977. Mass production of natural enemies, pp. 183-217. *In Biological Control by Augmentation of Natural Enemies: Insect and Mite Control with Parasites and Predators* (R. L. Ridgeway and S. B. Vinson Ed.), Plenum Press, New York.
- Morrison, R. K. 1985. *Chrysopa carnea*, pp. 419-426. *In Handbook of Insect Rearing* Vol. I (P. Singh and R. F. Moore Ed.), Elsevier, New York.
- Palmer, D. J. 1985. *Brachymeria intermedia*, pp. 383-393. *In Handbook of Insect Rearing* Vol. I (P. Singh and R. F. Moore Ed.), Elsevier, New York.
- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988. Mealybug wilt, mealybugs, and ants of pineapple. *Plant Disease* 72: 558-565.
- Sagarra, L. A., C. Vincent and R. K. Stewart. 2000. Fecundity and survival of *Anagyrus kamali* (Hymenoptera: Encyrtidae) under different feeding and storage temperature conditions. *European Journal of Entomology* 97: 177-181.

- Sakimura, K. 1939. List of natural enemies of pineapple pests imported and liberated by the experiment station of the pineapple producers cooperative association during the period 1930-1938. *Proceedings of the Hawaiian Entomological Society* 10: 352.
- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- van den Bosch, R., P. S. Messenger and A. P. Gutierrez. 1982. *An Introduction to Biological Control*. Plenum Press, New York, 247 pp.
- Venkatesan, T., S. P. Singh and S. K. Jalali. 2000. Effect of cold storage on cocoons of *Goniozus nephantidis* Muesebeck (Hymenoptera: Bethyridae) stored for varying periods at different temperature regimes. *Journal of Entomological Research* 24: 43-47.
- Whitaker-Deerberg, R. L., G. J. Michels, L. E. Wendel and M. Farooqui. 1994. The effect of short-term cold storage on emergence of *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) mummies. *Southwestern Entomologist* 19: 115-118.

**VIII. THE IMPACT OF BIG HEADED ANT ON *ANAGYRUS ANANATIS*  
GAHAN (HYMENOPTERA: ENCYRTIDAE) PARASITISM OF THE PINK  
PINEAPPLE MEALYBUG**

**INTRODUCTION**

Pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae), is one of the most widely distributed mealybugs in the tropics worldwide (Williams and Watson 1988). PPM and the closely related gray pineapple mealybug (GPM), *D. neobrevipes* Beardsley, can vector pineapple mealybug wilt associated virus (Sether *et al.* 1998), thereby leading to the expression of mealybug wilt disease of pineapple. Thus, both mealybugs are considered as the most important arthropod pests of pineapple in Hawaii and other locations (Rohrbach *et al.* 1988; German *et al.* 1992; Beardsley 1993; Hughes and Samita 1998).

At least six biological control agents of pineapple mealybugs (i.e., the parasitoids: *Anagyrus ananatis* Gahan, *Euryrhopalus propinquus* Kerrich, and *Hambletonia pseudococcina* Compere (Hymenoptera: Encyrtidae); and the predators: *Nephus bilucernarius* Mulsant, *Sticholotis ruficeps* Weise (Coleoptera: Coccinellidae) and *Lobodiplosis pseudococcidae* Felt. (Diptera: Ceceidomyiidae)) imported during the 1930's have successfully established in Hawaii (Gonzalez-Hernandez *et al.* 1999b).

Three species of ants (i.e., big headed ant (BHA), *Pheidole megacephala* F., Argentine ant, *Linepithema humile* (Mayr), and fire ant, *Solenopsis geminata* (F.) (Hymenoptera: Formicidae)) tend pineapple mealybugs (Reimer *et al.* 1990). BHA is the most common and most aggressive ant species in Hawaii below 600 m, where most of the

pineapple plantings are located (Reimer *et al.* 1990; Reimer 1994). When ants feed on honeydew, they protect the mealybugs against their natural enemies and also help them disperse from one plant to another (Nixon 1951; Buckley 1987; Petty and Tutsin 1993). Ant interference in the activities of the biological control agents of pineapple mealybugs is so prominent that ant control is absolutely necessary to achieve effective mealybug control (Petty and Tutsin 1993; Reimer and Gonzalez-Hernandez 1993). Ant predation of *N. bilucernarius* eggs and larvae affects the coccinellid's numerical response (Gonzalez-Hernandez 1995). Interference in the ovipositional behavior of *A. ananatis* affects its functional response (Gonzalez-Hernandez 1995; Gonzalez-Hernandez *et al.* 1999a).

Diazinon, a broad spectrum organophosphate insecticide, is the only registered insecticide for pineapple mealybug suppression in Hawaii (Rohrbach *et al.* 1988; HDOA 2001), which may interfere with the activities of mealybug natural enemies. The ant bait insecticide, Amdro® (Hydramethylnon, American Cyanamid Inc), which is permitted for emergency applications (Section 18) (HDOA 2001), effectively eliminates certain species of ants, thereby permitting the introduced agents to biologically control the mealybugs (Beardsley *et al.* 1982; Reimer and Beardsley 1990). However, this compound has never been registered for use in Hawaii and governmental approval for its use could be denied at any time. Additionally, Amdro can lose its effectiveness if not stored correctly. Within the last few years, control failures on Maui have been reported following the use of deteriorated Amdro. Lastly, organic production of pineapple is gaining a foothold in Hawaii, and no effective organic control methodologies are available for ant-infested

pineapple plantings. Therefore, alternative controls are needed for PPM and GPM suppression to allow growers some flexibility in their control choices.

Most introduced mealybug pests are effectively controlled by the introduction of their respective natural enemies (DeBach and Rosen 1991; Sagarra and Peterkin 1999; Neuenschwander 2001). Pineapple mealybugs, however, are among the most difficult pests to manage by the introduction of natural enemies due to the interference of ants (Bartlett 1978). Applications of diazinon may also interfere with natural enemy activities. Augmentative biological control by repeated field releases of mass reared parasitoids and predators would avoid the time lag in the natural enemies' numerical response (Huffaker *et al.* 1977). Encouraging results have been obtained with the augmentative biological control of some mealybugs. Releases of larval or adult mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), have resulted in as much as a 78% population reduction of the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homopteran: Pseudococcidae), on grape (Babu and Azam 1989). Augmentative field releases of *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) largely decreased citrus mealybug, *Planococcus citri* (Risso), infestations on citrus and custard apple in Queensland, Australia (Smith 1991). The potential of pineapple mealybug control by natural enemy augmentation has never been evaluated.

*Anagyrus ananatis*, is a highly specific, solitary endoparasitoid of PPM imported from Brazil during 1935-37 (Carter 1937). It was the most common parasitoid of PPM recovered during recent surveys in the Hawaiian pineapple agroecosystem (Gonzalez-Hernandez *et al.* 1999b). Caged field studies by Gonzalez-Hernandez *et al.* (1999b)

indicated that in the absence of ants *A. ananatis* would suppress PPM populations. Because of its relative abundance, host specificity, and persistence to attack PPM in the presence of ants, *A. ananatis* was considered as a candidate for augmentative biological control of PPM (Gonzalez-Hernandez 1995). However, laboratory studies by Gonzalez-Hernandez (unpublished data) indicated that BHA interferes in the ovipositional behavior of *A. ananatis*, reducing its effectiveness by as much as 60 percent. If augmentative releases are to be successful in the presence of ants, information is needed on the increased numbers of *A. ananatis* females that must be released to overcome BHA interference in PPM parasitization by *A. ananatis* as compared to conditions when ants are absent.

The main objective of the study reported here was to evaluate whether elevated release rates of *A. ananatis* could potentially overcome ant interference and provide effective suppression of PPM by parasitization. Studies were conducted in the laboratory to enable effective control over the numbers of parasitoids and ants used in the study.

## MATERIALS AND METHODS

PPM and *A. ananatis* individuals used in this experiment were obtained from cultures maintained at the Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, Hawaii. PPM was laboratory-reared on Kobocho squash using the methodology described in Chapter 3. *Anagyrus ananatis* individuals used in these studies were collected from Kunia, Oahu, HI, in October 1998. Since initial collection, *A. ananatis* was reared on mature PPM. Mated 2-3 day-old females were used in experimental studies. Prior to use in the experiments, females were

provided with honey and water and were exposed to PPM adults for about 24 hours to gain ovipositional experience. *A. ananatis* females were only used once in an experimental replication.

BHA colonies were collected from the University of Hawaii Pineapple Research Station at Whitmore, Oahu, and Dole Pineapple Plantation located in central Oahu, Hawaii. Individual colonies of ants were placed within a plastic shoe box (30 cm long x 15 cm wide x 9 cm high). Walls of the shoe box were coated with Fluon AD-1® (Norton Products Inc. Woodstock, RI) to prevent ant escape. Ants were provided with artificial nest chambers (90 mm diameter petri dish with about 5 mm thick plaster of Paris at the bottom) and a variety of food materials (e.g., pure honey, boiled eggs, dead flies and peanut butter) and water. Each colony had 2-3 queens and a large number of workers (> 500) and developing brood.

Pineapple leaves were collected from six month-old potted plants ( Smooth Cayenne cv. Champaka 153, Fresh Delmonte Hawaii, Inc.) grown in the greenhouse. Leaves were trimmed to a length of 25 cm such that about 5 cm of the basal portion of the leaf consisted of the succulent whitish area. Both cut ends of the leaf were coated with molten wax. About eighty PPM adults were transferred to the pineapple leaves and allowed to settle for one day before they were used in the experiment.

The experimental arena consisted of a plastic shoe box (30 cm long x 15 cm wide x 9 cm high) covered with a transparent Plexiglas panel. One PPM infested leaf was placed in each arena. The experiment was conducted in a 2 x 4 factorial design. The first factor was the presence of ants (a shoe box with an ant colony was connected to the arena box with a 5 mm internal diameter plastic tube) or absence of ants (control, not connected



to any ant colony) (Fig. 8.1). The second factor was the density of *A. ananatis* (one, two, four and eight females) per arena cage.

The numbers of BHA workers tending mealybug individuals within the experimental arenas varied dramatically throughout the day and among ant colonies. Thus, treatments were assigned in such a manner that each of the four ant colonies had an opportunity to protect the mealybugs against each of the four *A. ananatis* density treatments used in the study (4 x 4 Latin square designs).

*Anagyrus ananatis* females were introduced through the entry tube two hours after the placement of the PPM-infested leaf into the experimental arena. Mealybugs were placed onto the cut pineapple leaves a day before the experiment began. On the day of the experiment, the leaves with the mealybugs were placed in the arena at 1300 Hours. At 1500 Hours, the *A. ananatis* females were introduced through the entry tube. The arena was placed in a temperature cabinet ( $24 \pm 1^\circ\text{C}$  and 50-55% RH). Light was provided for the initial 14 hours, after which a 6-hour scotophase period began. On the following day, the light came on at 1100 Hours and the mealybugs on the pineapple leaf were immediately removed and the ants were counted. All the ants present in the arena were returned back to the box containing their respective ant nests. The PPM-infested pineapple leaf and all the mealybugs were transferred to a 35 x 15 cm plastic bag and held at  $24 \pm 1^\circ\text{C}$ . After 14 days, all the mealybugs were examined under a microscope, and mealybug mummies found were placed in individual gel capsules (Size 2).

The first set of experiments was conducted in January 2002 with 8 replications. A considerable number of mealybugs were removed from the arena by the ants. In the second experimental setup (12 replications), the end of the tube, which connected the

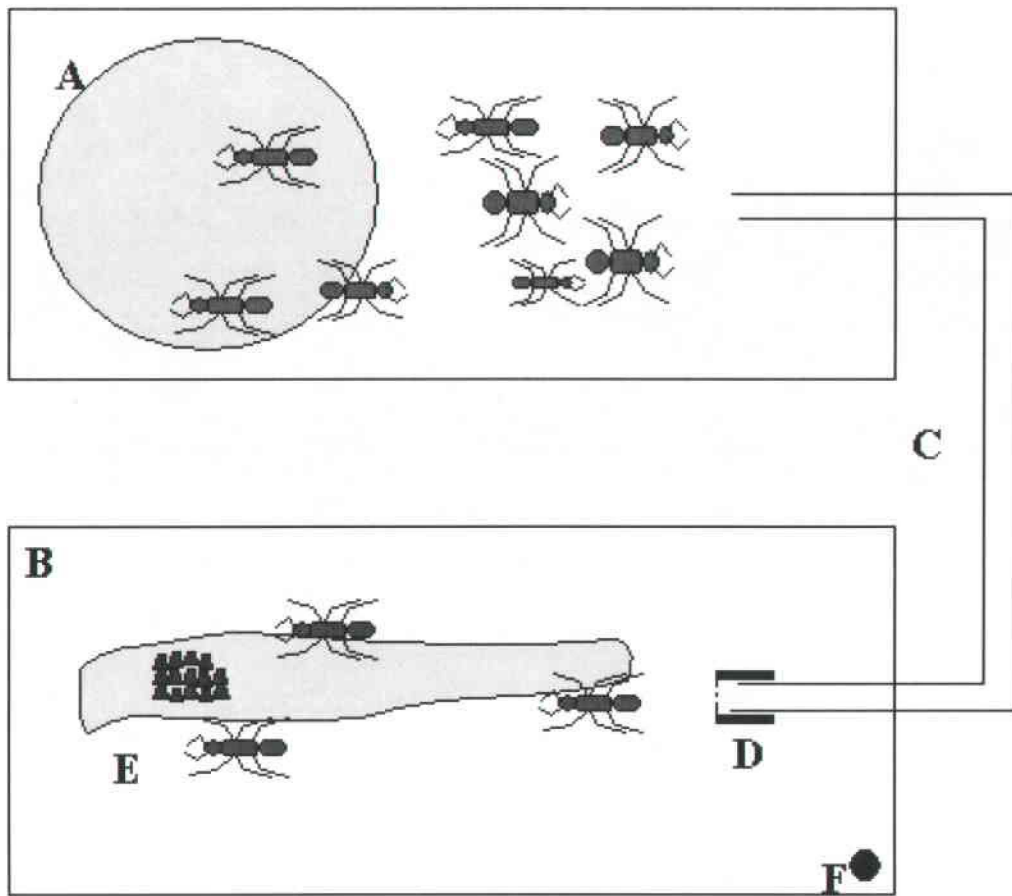


Fig. 8. 1. Schematic diagram showing experiment setup. (A) big headed ant colony, (B) experiment arena, (C) plastic tube, (D) fine mesh cap, (E) mealybugs on pineapple leaf, (F) *Anagyrus ananatis* entry tube. The control arena was not connected to any ant colony.

arena and ant colony, was fitted with a fine mesh (12 mesh per cm) barrier which permitted the movement of ants alone, but not with those carrying a mealybug.

*Statistical Analysis:* Percent parasitization in each experimental arena was calculated based on the number of mummified mealybugs and the total mealybugs recovered. The data were arcsine square root transformed and analyzed by ANOVA procedures (SAS Institute 1999).

## RESULTS AND DISCUSSION

*Foraging Ants:* In the first set of replications (January 2002), with respect to the ant colonies, the mean foraging ant densities ranged from  $13.2 \pm 1.5$  to  $27.5 \pm 3.25$  workers / soldiers and they were significantly different among colonies ( $F_{3,25} = 8.91$ ,  $P = 0.0003$ ). Within the experimental treatments, the mean ant densities tending the mealybugs exposed to the various *Anagyrus* densities ranged from  $12 \pm 2.3$  to  $20.1 \pm 3.6$  and they were also significantly different ( $F_{3,25} = 3.32$ ,  $P = 0.036$ ). The density treatment with four parasitoids per arena cage had significantly fewer attending ants ( $12.0 \pm 2.3$ ) compared to  $19.1 \pm 2.7$ ,  $20.1 \pm 3.6$  and  $20.9 \pm 3.3$  ants for one, two and eight females per arena cage, respectively.

In the second set of replicates, after which a barrier was installed to prevent mealybug removal, the number of ants attending the mealybugs in the experimental arena was higher than in the first set of replications ( $t_{56} = 7.5$ ,  $P < 0.0001$ ). The mean numbers of ants foraging in the experimental arena in the second set of replications ranged from  $38.2 \pm 7.8$  to  $79.7 \pm 8.1$  individuals for the four ant colonies used, and they were again significantly different ( $F_{3,41} = 3.77$ ,  $P = 0.0177$ ). The mean number of ants tending the

PPM in the various *Anagyrus* density treatments ranged from  $46.7 \pm 7.0$  to  $73.7 \pm 12.7$  individuals, but they were not significantly different ( $F_{3,41}=1.52, P = 0.200$ ). The use of the screen on the tube apparently discouraged the ants from returning to the colony once they entered the arena, thus leading to higher numbers of foragers in the second set of replications.

*Mealybug Recovery:* There was a significant interaction effect regarding the number of mealybugs recovered between the two sets of replication and ant attendance ( $F_{1,146} = 34.5, P < 0.0001$ ). In the first replication set, moisture accumulation from the pineapple leaf within the plastic bag affected mealybug survival and resulted in the loss of about 5 - 10% of the mealybugs in all treatments. In this replication set, the treatments with ants had significantly fewer mealybugs recovered than the control without ants ( $F_{1,146} = 98.7, P < 0.0001$ ) (Table 8.1).

When the screen barrier was used at the end of the connecting tube in the second set of replications, almost all of the mealybugs exposed in the arena were recovered. More mealybugs were recovered in treatments lacking ants ( $F_{1,146} = 42.2, P < 0.0001$ ). *Anagyrus ananatis* density did not have any effect on the rate of recovery of mealybugs at the end of the experiment ( $F_{3,146} = 0.8, P = 0.5268$ ). No interactions were found between the presence of ants ( $F_{3,146} = 0.3, P = 0.7987$ ) or the barriers ( $F_{3,146} = 0.5, P = 0.6535$ ) and the numbers of *A. ananatis* females. These results show that BHA transports PPM, and they provide additional evidence for the long suspected belief that pineapple mealybugs are dispersed by ants in the field (Illingworth 1931; Carter 1932). This is contrary to the findings of Jahn and Beardsley (1996) that reported lack of evidence that ants dispersed GPM.

Table 8. 1. Effect of ant attendance and screen barrier on PPM recovery<sup>a</sup>.

<i>Anagyrus</i> density	Without barrier		With barrier <sup>b</sup>	
	Ants Present	Ants Absent	Ants Present	Ants Absent
1	59.5 ± 6.0	72.6 ± 2.5	79.6 ± 0.5	80.2 ± 0.2
2	57.7 ± 5.6	76.6 ± 1.8	78.8 ± 0.8	80.2 ± 0.2
4	60.4 ± 6.4	75.7 ± 2.5	80.0 ± 0.3	80.0 ± 0.3
8	64.5 ± 3.7	76.6 ± 1.1	79.7 ± 0.7	80.7 ± 0.3

<sup>a</sup> 80 adult PPM originally placed in experimental arenas

<sup>b</sup> Mesh barrier placed on tube to prevent removal of PPM by ants.

*Parasitization of PPM:* There was no significant interaction among the presence of the ant barriers, ant attendance and *A. ananatis* density. Ants effectively reduced PPM parasitization to almost half of that recorded on mealybugs in their absence ( $F_{1,146} = 18.2, P < 0.0001$ ) (Table 8.2). Increasing the *A. ananatis* density significantly increased the parasitization rate ( $F_{3,146} = 27.2, P < 0.0001$ ). The mean parasitization rate increased from 5.4 to 33% when the *A. ananatis* density was increased from one female to eight females per arena cage, respectively, in the absence of ants. When ants were present, the PPM parasitization rate ranged from 4.3 to 20.3% from one to eight *A. ananatis* females per arena cage, respectively.

In this study, the parasitization rate achieved by introducing eight female parasitoids in the presence of ants was comparable to the parasitization rate from the introduction of only four female parasitoids in the absence of ants (Table 8.2). These results also showed that increases in *A. ananatis* densities increased the PPM parasitization rates and suggested that augmentative field releases of the laboratory reared *Anagyrus* may aid to suppress PPM in the presence of ants.

The mean reduction in the parasitization rate due to ant interference in this study ranged from 18 to 56% compared to the respective control, which is comparable to the previously reported 48% reduction of PPM parasitization by *A. ananatis* due to ant interference (Gonzalez-Hernandez *et al.* 1999a). The comparable trend of parasitization in the two replication sets, despite the removal of a large number of mealybugs in the first replication set, suggests that the ants did not discriminate parasitized mealybugs from healthy mealybugs, within 24 hours of parasitization, when selecting mealybugs to take to the ant colony. Further investigation is necessary to determine whether ants would be

Table 8. 2. Effect of ant attendance and female *A. ananatis* density on percentage PPM parasitization .

Female <i>Anagyrus</i> density	Percentage PPM Parasitization <sup>a</sup>						Mean % reduction <sup>c</sup>
	Without barrier		With barrier		Pooled		
	Ants Present	Ants Absent <sup>b</sup>	Ants Present	Ants Absent <sup>b</sup>	Ants Present	Ants Absent <sup>b</sup>	
1	3.6 ± 1.9	5.5 ± 1.7	4.8 ± 2.5	5.4 ± 1.3	4.3 ± 1.6	5.4 ± 1.0	20.4
2	10.3 ± 3.8	8.1 ± 2.1	10.1 ± 1.9	15.2 ± 2.2	10.1 ± 1.9	12.4 ± 1.7	18.5
4	9.2 ± 3.3	18.7 ± 5.9	8.1 ± 2.4	20.9 ± 3.8	8.8 ± 1.9	20.0 ± 3.2	56.0
8	16.4 ± 4.7	29.7 ± 8.0	22.7 ± 3.2	35.0 ± 3.8	20.2 ± 2.7	32.9 ± 3.9	38.6

Data were arcsine square root transformed and analyzed with ANOVA procedures.

<sup>a</sup> 80 PPM adults initially present in arena

<sup>b</sup> Control

<sup>c</sup> Mean percent reduction in PPM parasitization in presence of ants compared the corresponding control without ants based on pooled data).

able to differentiate a parasitized mealybug as parasitoid development advances within the mealybug. However, recovery of PPM mummies from ant infested pineapple plants in commercial pineapple plantings by Gonzalez-Hernandez *et al.* (1999b) and Pandey (unpublished data) suggests that parasitoids can survive in the mummies among the ant-tended mealybugs.

Because ants are commonly associated with honeydew-producing homopterans, their effects on the activities of natural enemies have been frequently documented. Although increases in the parasitization rates of *Aphis fabae* (Scop.) (Homoptera: Aphididae) by *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Aphelinidae) in ant-tended colonies have been reported (Volkl and Stechmann 1998), ant tending generally interferes with the activities of biological control agents. Significantly fewer egg masses of *Catantopiniella rugosa* (Heteroptera: Plataspidae) were parasitized on ant-occupied trees than on ant-excluded trees (Gibernau and Dejean 2001). Galls of *Disholcaspis edura* Weld (Homoptera: Psyllidae) on *Quercus turbinella* showed that when ants tended galls, the rate of parasitism by *Platygaster* sp. was reduced to about 30% compared to 51% when ants were excluded (Fernandes *et al.* 1999). Stechman *et al.* (1996) found only *Micromus timidus* (F.) (Neuroptera: Hemerobiidae) as a predator of ant-tended banana aphids, *Pentalonia nigronervosa* Coquerel (Homoptera: Aphididae), while syrphids and coccinellids were regularly encountered in the ant-free colonies. Most important was the reduction of *Aphis gossypii* Glover (Homoptera: Aphididae) parasitism by the aphelinid *Aphelinus gossypii* Timberlake (Hymenoptera: Aphelinidae) in the presence of ants. Also, the introduced aphid parasitoid *Aphidius colemani* Viereck



(Hymenoptera: Braconidae) was heavily attacked by various ant species (Stechman *et al.* 1996).

Ant attendance was critical for the survival of pineapple mealybugs (Illingworth 1931; Carter 1933; Jahn and Beardsley 1994) and suppression of ants by ant baits led to a decrease in mealybugs, primarily due to the activities of biological control agents (Gonzalez-Hernandez *et al.* 1999a). Previous field surveys showed that the mean PPM density ranged from 23 to 157 individuals per plant and mean number of *A. ananatis* reared from each plant ranged from 0.1 to 5.1 individuals (Gonzalez-Hernandez 1995). The natural enemy densities were lower in the ant-infested plots than the ant-free plots and their activities appeared much later in the ant-infested plots than the ant-free plots (Gonzalez-Hernandez 1995).

Under standard pineapple planting densities (plant to plant distance 20 to 25 cm within lines, two lines 40 cm apart within a row, and 90 cm spacing between rows) plant density may vary from about 61,000 to 77,000 plants per hectare (Py *et al.* 1987). About 4 parasitoids (male + female) were reared per plant in the ant free plots after 56 days of bait application (to control ants) which eventually led to the suppression of PPM in those plots (Gonzalez-Hernandez 1995). Based on the results of the laboratory studies reported here, to achieve a similar level of PPM suppression under ant infested conditions, it may require releases of about eight parasitoids per plant (= 500,000 to 600,000 individuals per hectare). Because PPM infestations begin from the edges of the field, treatments of smaller areas of pineapple with augmentative field releases may be adequate making the augmentative biological control a viable option.

## REFERENCES

- Babu, T. R. and K. M. Azam. 1989. Biological control of grape mealybug *Maconellicoccus hirsutus* (Green). Indian Journal of Plant Protection 17: 123-126.
- Bartlett, B. R. 1978. Pseudococcidae, pp. 137-171. In Introduced Parasites and Predators of Arthropod Pest and Weeds: A World Review (C. P. Clausen Ed.), U. S. Department of Agriculture, Handbook No. 480 Washington D.C.
- Beardsley, J. W., T. H. Su, F. L. McEwen and D. Gerling. 1982. Field investigations on the interrelationships of the big headed ant, the gray mealybug and the pineapple wilt disease in Hawaii. Proceedings of the Hawaiian Entomological Society 24: 51-67.
- Beardsley, J. W. 1993. The pineapple mealybug complex: taxonomy, distribution and host relationships. pp. 383-386. In "Acta Horticulturae 334" (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First International Pineapple Symposium, 2-6 November, 1992 Honolulu, Hawaii.
- Buckley, R. 1987. Interaction involving plants, homoptera and ants. Annual Review of Ecology and Systematics 18: 111-135.
- Carter, W. 1932. Border plantings as guard rows in pineapple mealybug control. Journal of Economic Entomology 25: 1027-1031.
- Carter, W. 1933. The pineapple mealybug *Pseudococcus brevipes* and wilt of pineapple. Phytopathology 23: 207-242.
- Carter, W. 1937. Importation and laboratory breeding of two chalcid parasites of *Pseudococcus brevipes* (Ckl.). Journal of Economic Entomology 30: 370-372.

- DeBach, P. and D. Rosen. 1991. *Biological Control by Natural Enemies*. Cambridge University Press, Cambridge, UK, 440 pp.
- Fernandes, G. W., M. Fagundes, R. L. Woodman and P. W. Price. 1999. Ant effects on three-trophic level interactions: plant, galls, and parasitoids. *Ecological Entomology* 24: 411-415.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. *Advances in Disease Vector Research* 9: 241-259.
- Gibernau, M. and A. Dejean. 2001. Ant protection of a heteropteran trophobiont against a parasitoid wasp. *Oecologia* 126: 53-57.
- Gonzalez-Hernandez, H. 1995. *The Status of Biological Control of Pineapple Mealybugs in Hawaii*. Ph.D. Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Gonzalez-Hernandez, H., M. W. Johnson and N. J. Reimer. 1999a. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15: 145-152.
- Gonzalez-Hernandez, H., N. J. Reimer and M. W. Johnson. 1999b. Survey of the natural enemies of *Dysmicoccus* mealybugs on pineapple in Hawaii. *BioControl* 44: 47-58.
- Huffaker, C. B., R. L. Rabb and J. A. Logan. 1977. Some Aspects of Population Dynamics Relative to Augmentation of Natural Enemy Action, pp. 3-38. *In* *Biological Control by Augmentation of Natural Enemies* (R. L. Ridgeway and S. B. Vinson Ed.), Plenum Press, New York.

- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.
- Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Jahn, G. C. and J. W. Beardsley. 1994. Big-headed ants, *Pheidole megacephala*: Interference with the biological control of gray pineapple mealybugs, pp. 199-205. *In Exotic Ants* (D. F. Williams Ed.), West View Press, Boulder, Colorado.
- Jahn, G. C. and J. W. Beardsley. 1996. Effects of *Pheidole megacephala* (Hymenoptera: Formicidae) on survival and dispersal of *Dysmicoccus neobrevipes* (Homoptera: Pseudococcidae). *Journal of Economic Entomology* 89: 1124-1129.
- Neuenschwander, P. 2001. Biological control of the cassava mealybug in Africa: A review. *Biological Control* 21: 214-229.
- Nixon, G. E. J. 1951. *The Association of Ants with Aphids and Coccids*. CIE, London, 36 pp.
- Petty, G. J. and H. Tutsin. 1993. Ant (*Pheidole megacephala* F.) - Mealybug (*Dysmicoccus brevipes* Ckll.) relationship in pineapples in south Africa. pp. 387-395. *In "Acta Horticulturae 334"* (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First international pineapple symposium, 2-6 Nov, 1992 Honolulu, Hawaii.
- Py, C., J. J. Lacoeylthe and C. Teisson. 1987. *The Pineapple Cultivation and Uses*. G. P. Maisonneuve et Larose, Paris, 568 pp.

- Reimer, N., J. W. Beardsley and G. Jahn. 1990. Pest ants in the Hawaiian Islands, pp. 40-50. *In* Applied Myrmecology. A World Perspective (K. vander Meer, K. Jaffe and A. Cedeno Ed.), West View Press, Boulder, Colorado.
- Reimer, N. J. and J. W. Beardsley. 1990. Effectiveness of hydramethylnon and fenoxycarb for control of bigheaded ant (Hymenoptera: Formicidae), an ant associated with mealybug wilt of pineapple in Hawaii. *Journal of Economic Entomology* 83: 74-80.
- Reimer, N. J. and H. Gonzalez Hernandez. 1993. Evidence for colony founding by haplometrosis in big-headed ant, *Pheidole megacephala*. pp. 397-405. *In* (D. P. Bartholomew and K. G. Rohrbach Ed.). Proceedings, First international pineapple symposium, Honolulu, HI.
- Reimer, N. J. 1994. Distribution and impact of alien ants in vulnerable Hawaiian ecosystems, pp. 11-22. *In* Exotic Ants (D. F. Williams Ed.), Westview Press, Boulder, Colorado.
- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988. Mealybug wilt, mealybugs, and ants of pineapple. *Plant Disease* 72: 558-565.
- Sagarra, L. A. and D. D. Peterkin. 1999. Invasion of the Caribbean by the hibiscus mealybug, *Maconellicoccus hirsutus* Green (Homoptera: Pseudococcidae). *Phytoprotection* 80: 103-113.
- SAS Institute. 1999. The SAS System for Windows release 8.0. SAS Institute, Cary, NC, USA.

- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.). *Phytopathology* 88: 1224-1230.
- Smith, D. 1991. The use of *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) to control *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) in custard apples in Queensland. *General and Applied Entomology* 23: 3-8.
- Stechman, D. H., W. Volkl and P. Stary. 1996. Ant-attendance as a critical factor in the biological control of the banana aphid *Pentalonia nigronervosa* Coq. (Hom.: Aphididae) in Oceania. *Journal of Applied Entomology* 120: 119-123.
- Volkl, W. and D. H. Stechmann. 1998. Parasitism of the black bean aphid (*Aphis fabae*) by *Lysiphlebus fabarum* (Hym., Aphidiidae): The influence of host plant and habitat. *Journal of Applied Entomology* 122: 201-206.
- Williams, D. J. and G. W. Watson. 1988. *The Scale Insects of Tropical South Pacific Region*. CAB International, Wallingford, 260 pp.

## IX. SURVEY FOR PINK PINEAPPLE MEALYBUG AND ASSOCIATED PARASITOIDS IN WEEDS BORDERING PINEAPPLE PLANTINGS

### INTRODUCTION

Pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell), and gray pineapple mealybug (GPM), *Dysmicoccus neobrevipes* Beardsley, (Homoptera: Pseudococcidae) are the most important mealybugs associated with pineapple crops worldwide (Beardsley 1959, 1965; Rohrbach *et al.* 1988; German *et al.* 1992). Their ability to vector pineapple mealybug wilt associated virus (PMWaV) and induce typical wilt symptoms have raised their status to that of the most important pineapple pest worldwide (Sether *et al.* 1998). From early on, these mealybugs were identified as the key factor in the mealybug wilt disease of pineapple (MWP) (Illingworth 1931). The PPM commonly infests the roots, leaves, stems, fruit and crowns of pineapple, whereas the GPM infests only the aerial roots, stems, fruit and crowns (Beardsley 1959; Rohrbach *et al.* 1988; Jahn and Beardsley 2000).

PPM attacks more than 140 plant species throughout the tropical and subtropical parts of the world. In contrast, GPM has a smaller geographic distribution, limited to South and Central America, Caribbean, some Pacific Islands and a few Asian countries bordering the Pacific Ocean, and infests about 50 plants species (Beardsley 1965; Williams and Watson 1988; Williams and Willink 1992; Ben Dov 1994). In addition to pineapple and other bromeliads, important hosts of PPM recorded from Hawaii were banana, *Musa x paradisiaca*. L. (Musaceae), sugarcane, *Saccarum officinarum* L., rhodes grass, *Chloris gayana* Kunth, natal grass, *Rhynchelytrum repens* (Willd.) Hubb.,

guinea grass, *Panicum maximum* Jacq., common sand burr, *Cenchrus echinatus* L., and vasey grass (*Paspalum urvelii* Steud.) (Poaceae) (Carter 1932a; Carter 1933a; Carter 1933b, 1951; Beardsley 1959). GPM has a more restricted host plant range, which includes century plant, *Agave sisalana* L. (Agavaceae) in addition to pineapple and bananas (Carter 1933a; Carter 1951).

Pineapple mealybugs can reproduce on vegetative plant parts such as crowns and suckers used for planting (Carter 1932a; Rohrbach and Apt 1986). Submersion of crowns and slips in an insecticide prior to transplant should effectively eliminate the mealybugs from planting materials (Jahn and Beardsley 2000). Weeds surrounding pineapple plantings are considered important mealybug hosts and sources of mealybug survival and infestation (Carter 1951; Rohrbach *et al.* 1988). Appearance of MWP along the edge of pineapple plantings (referred to as “edge wilt” in the early literature) also suggested the movement of mealybugs from the weeds into the crop (Carter 1932a). Attending ants and/or the wind were considered the responsible agents of dispersal of pineapple mealybugs (Carter 1932a; Jahn and Beardsley 2000). Before the availability of highly effective insecticides, treated sunken boards as well as pineapple guard rows were commonly utilized to interfere with the movements of ants carrying mealybugs into pineapple crops (Carter 1932b).

Plastic mulch laid over pineapple rows conserves moisture and facilitates fumigation against nematodes while also suppressing weeds within plantings (Rohrbach and Apt 1986). However, pineapple plantations are commonly surrounded by dense patches of weeds. Commercial pineapple plantings in Hawaii frequently extend from paved highways to natural grassy or forested areas. Weeds are most commonly observed



along the highways adjacent to pineapple plantings where plant communities are often “disturbed” due to weed management practices (e.g., mowing, herbicide applications, burning) and “undisturbed” along the uncultivated borders of the plantations where weed control is not practiced. Many pineapple plantings are completely encircled by dirt roads in which no weeds are allowed to grow, thus only specifically located plantings, usually along the outside edges of the plantations or along steep gully / ditch banks, have weedy borders. Weeds such as guinea grass, sour grass, *Digitaria insularis* (L.) Mez ex Ekman (Poaceae), and century plant are commonly found scattered within pineapple plantings, mostly in the ratoon crops as well as some ditches within plantations (RRP, personal observation). However, the relative importance of various weed species, which border plantings, as reservoirs and sources for mealybugs has not been quantified. Similarly, several predators and parasitoids attack mealybugs in pineapple, but their association with mealybug populations infesting weeds is unknown.

Several natural enemies have been reported attacking PPM and GPM in pineapple (Gonzalez-Hernandez *et al.* 1999). *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae), a highly host specific parasitoid (Noyes and Hayat 1994), is the dominant natural enemy of PPM in Hawaii’s pineapple crops. PPM is the most preferred host of *A. ananatis* reported from Hawaii (Gonzalez-Hernandez 1995). Unfortunately, no information is available on *A. ananatis* in other cropping systems or weeds surrounding pineapple crops. Additionally, the local origins of *A. ananatis* individuals that disperse into new pineapple crops to parasitize new PPM infestations are unknown, and this information may potentially increase our abilities to manipulate populations of *A. ananatis* for enhanced PPM suppression.

The objectives of the field surveys reported here were to: 1) identify and quantify the composition of plant flora in disturbed and undisturbed areas bordering Oahu pineapple plantings; 2) evaluate the weedy plants bordering pineapple plantings as potential sources of PPM infestations for adjacent pineapple plantings; and 3) document the presence of the PPM parasitoid *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae) among mealybugs infesting the plants around pineapple plantings.

## MATERIALS AND METHODS

The study was conducted in three parts. Initial surveys were conducted to identify the general presence and distribution of weedy plants and *Dysmicoccus* mealybugs, within undisturbed and disturbed areas, bordering pineapple plantings, that support weed growth. Based on those results, surveys were conducted in disturbed areas bordering plantings to quantify weed diversity therein. Lastly, a survey was focused on rhodes grass, which was the most common PPM infested weed.

*Locating mealybug infested sites.* Pineapple plantings were surveyed on Oahu's central plain, near Waihiawa and Kunia, between the ranges of the Waianae Mountains and the Koolau Mountains. A total of 34 "disturbed" sites were checked along Kamehameha Highway (Hwy 99) (14 sites), Wilikina Drive (8 sites), Kunia Road (5 sites), and Kaukonahua Road (9 sites); and eight "undisturbed" sites along an unpaved farm road on the Del Monte Plantation at Kunia, Field No. 2095 (4 sites) and Field No. 2090A (4 sites) during July 19 – 21, 1999. On each roadway, the sampled sites were 0.5 km distance from each other. The first site in a group of sites along any given road was selected by picking a random point along the roadway, and proceeding 0.5 km away from

the initial point. Additional sites along the road were at 0.5 km intervals away from the initial randomly selected point.

Pineapple plantings and weeds adjacent to the plantings were assessed for the presence of mealybugs. At each site, at least ten pineapple plants and the major weed species present were examined for the presence of mealybugs. Because mealybugs were not present at all sites, further efforts were focused on those locations with mealybug infestations.

*Sampling mealybug infested sites.* Two sites (Dole Food Company, Hawaii, Field No Kemoo-1 and Kemoo-3) along Kaukonahua Road and one site (Fresh DelMonte Hawaii, Field No. 3013) on Kunia Road were selected for a detailed vegetation survey within the disturbed areas between the pineapple field border and paved highway. At each site, four one meter wide transects were surveyed to obtain data on the weed species present, their densities, and the mealybug densities present on each weed species. Each transect began at the edge of the pineapple planting border and extended perpendicularly towards the paved highway. The transect consisted of two to three 1 m<sup>2</sup> quadrats. From the planting border, the first quadrat extended from 0 to 1 m; the second quadrat extended from 2 to 3 m; and the third quadrat extended from 4 to 5 m. The areas between quadrats 1 and 2 and quadrats 2 and 3 were not inspected. When the weeds within a quadrat were more than 30 cm tall, they were clipped about 25 cm above the soil surface with a hedge shear before the quadrat was placed on the ground. Weed densities were measured by a point quadrat (25 pins per m<sup>2</sup>) frame (Fig. 9.1). The first transect was randomly established at a 2 m distance from the front bumper of the researcher's vehicle.

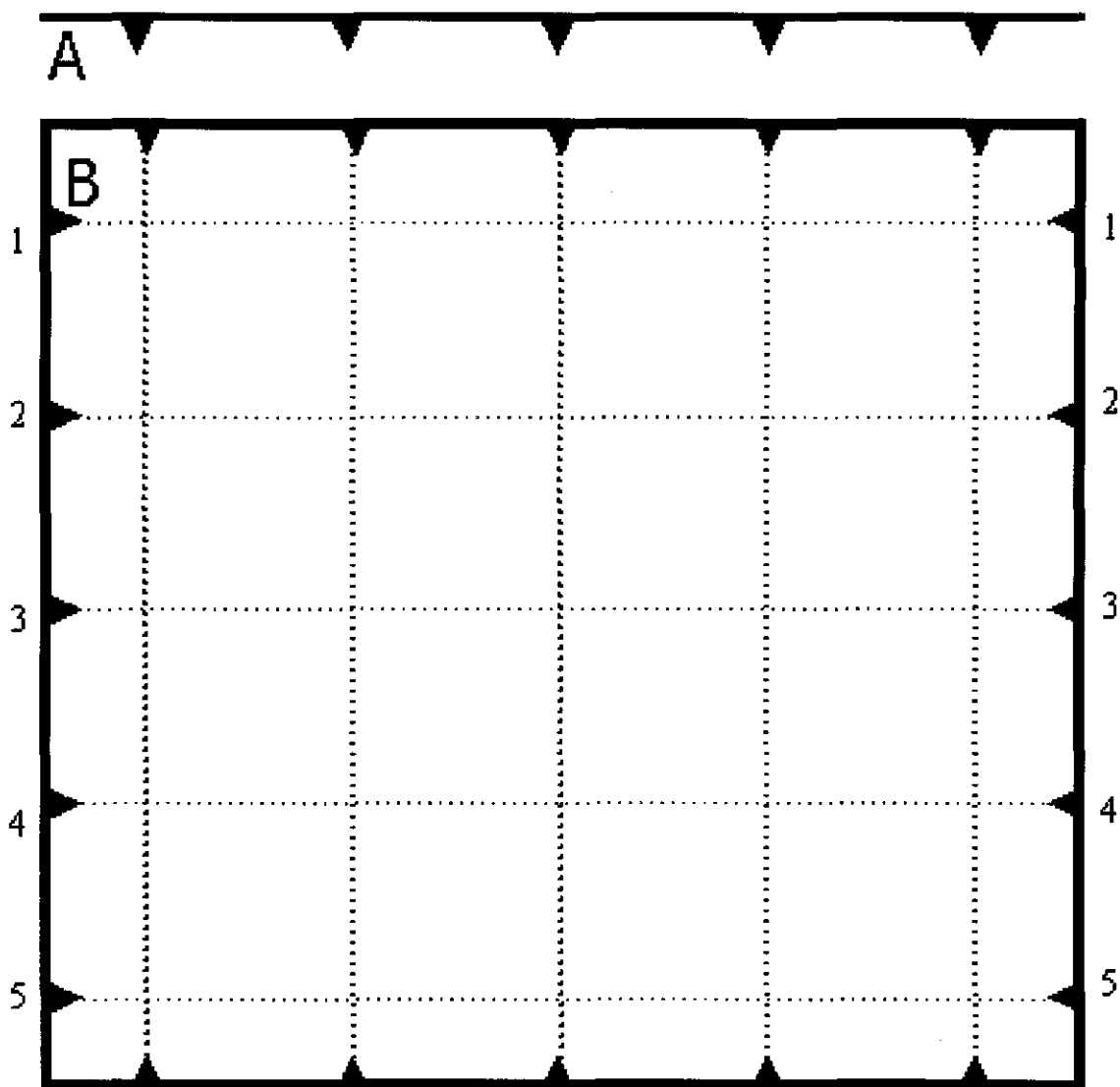


Fig. 9. 1. Twenty-five pin point-quadrat. (A) Wooden pole fitted with 5 pins 20 cm apart. (B) 1 m x 1 m frame marked on all sides at 20 cm. The wooden pole was placed over the frame at points from 1 to 5 and vegetation pointed to or touched by the pin recorded.

Placement of the remaining transects was established at about 9 m intervals to the south or east of the initial transect.

For each pin suspended within the quadrat frame (Figure 9.1), information was recorded relative to the weed species, if any, in contact with the pin. After estimating weed densities, five to ten plants of each major weed species within each transect were examined for the presence of mealybugs. Mealybug density was recorded by counting all the stages except the crawlers. Adult mealybugs were collected in clear gelatin capsules (size 2), transported to the laboratory, and held for parasitoid emergence at room temperature and  $50 \pm 5$  % RH.

One site (Dole Food Company, Hawaii Field No. Whitmore 2) in an undisturbed area was also surveyed following the procedures described above. However, observations were only recorded from two quadrats because the border area extended into a deep gulch, which had similar vegetation as in the second quadrat.

The frequency distributions of the various weeds in each quadrat were transformed to percentages and the means calculated to quantify the commonness of each weed species. The number of mealybugs per plant per weed species was estimated by calculating the mean from the number of plants examined in all the sites.

*Determination of Weed Diversity.* Based on the findings of the preliminary survey, two field surveys were conducted during 15 – 18 August 2000 and 1 – 4 January 2001. The survey methodology was modified so that more sites could be surveyed and data on weeds as well as mealybug prevalence could be collected more efficiently. From randomly determined starting points, a total of 39 sites were surveyed in August 2000 along Kamehameha Highway (20 sites), Kaukonahua Road (5 sites), and Wilikina Drive

(14 sites) at 0.5 km intervals. Similarly, 38 sites along Kamehameha Highway (4 sites), Kaukonahua Road (14 sites), Kunia Road (10 sites), and Wilikina Drive (14 sites) at 0.5 km intervals in January 2001.

Weeds closer to the paved roadways were regularly mowed (hereafter called “mowed band”) and their growth was limited. Further away from the roadways and closer to the pineapple field borders, the weeds were not mowed, and grew taller and denser (hereafter called “unmowed band”). Widths of the mowed and unmowed bands were measured perpendicular to the edges of the pineapple plantings at each sample site. A quadrat frame (0.1 m<sup>2</sup>) was randomly tossed ten times each in the mowed and unmowed areas at each site. All weed species found within the quadrat were recorded and examined for the presence of PPM. The number of plants of rhodes grass, natal grass, and wire grass, *Eleusine indica* (L.) Gaertn. (Poaceae), within the quadrat were counted. When the plants were infested, the number of adult and third instar mealybugs per plant were counted, and collected in the gelatin capsules (Size 2) and held for parasitoid emergence in the laboratory.

The density of each weed species was calculated as the mean number of weed species recorded per site (1 m<sup>2</sup>). Mean species density for the two bands (mowed and unmowed) and two survey seasons were compared by ANOVA procedures. The relative abundance of a weed species was expressed as its frequency relative to the total sites surveyed for each band in each season.

*Mealybug Densities on Rhodes Grass:* Rhodes grass was the most common weed host of PPM recorded in surveys, and attempts were made to estimate the PPM densities on the plant. Two locations along the pineapple growing areas with high mealybug

infestations on rhodes grass were identified. Thirty five plants from Kamehameha Highway site and 25 plants from Helemano Plantation Site (Dole Food Hawaii, Inc.) were carefully removed and examined for numbers of second and third instars and adult PPM per plant. The total numbers of tillers as well as the tillers at various growth stages (e.g., vegetative stage: no evident sign of flower development; booting: flower head enclosed within a swollen leaf sheath; flowering: anthers protruding out on the flower head; seed maturity: seed formation; seed dispersal: few seeds have dropped from the tip of the rachis; and dying head: seed head fallen off) from each plant were also counted. Adult mealybugs were collected individually in clear gelatin capsules (Size 2) and held in the laboratory for parasitoid emergence.

Correlation analysis was performed to determine the relationship between the number of mealybugs found per plant and the plant characteristics (plant height, number of tillers at various growth stages) using the data from the mealybug-infested plants. Regression analysis was performed to evaluate the possibility of predicting mealybug density per plant based on the plant's characteristics.

Whether the mealybugs infesting rhodes grass were suitable for parasitization by *Anagyrus ananatis* was determined by exposing the mealybugs collected on rhodes grass to *A. ananatis*. Mealybugs were collected from rhodes grass from four locations (Manoa, Poamoho, Ewa and Kunia) on Oahu. Half of the collected mealybugs from each site were exposed to adult *A. ananatis* for 48 hours and the remaining half were not exposed (control). The number of mummies was counted after 14 days.

## RESULTS

Plants were tall (> 2 m height) and mature in the undisturbed areas surrounding pineapple production areas away from the paved roadways. Guinea grass and paragrass, *Brachiaria mutica* (Forssk.) Stapf (Poaceae), were found in these areas and guinea grass was the dominant species. The guinea grass covered the ground surface within the first quadrat of the first transect and all the quadrats of the remaining three transects.

Interestingly, when the guinea grass was clipped to 25 cm in height, 88/175 of the pins pointed to bare ground, whereas only 87/175 pins pointed to the guinea grass stumps. In the first transect, paragrass completely covered the ground surface in the second quadrat and beyond. No mealybugs were found in these transects.

In the disturbed areas near the paved roadways, the weeds were shorter near the road and taller near the pineapple crop. Although growth of foliage apparently covered the space, clipping the weeds at 25 cm height exposed the ground. More than one third of the pins pointed to bare ground (first quadrat, adjacent to pineapple crop) (Table 9.1). Bare soil was less prevalent as the distance from the pineapple border increased. In the second quadrat about 18% of the pins pointed to bare ground and less than 1.5% of the pins indicated bare ground in the third quadrat. *Chloris gayana*, *Digitaria insularis*, *Rhynchelyrum repens*, *Euphorbia* sp. L. (Euphorbiaceae), *Panicum maximum* and *Ipomea* sp L. (Convolvulaceae) were more common within the 1 to 2 m from the pineapple border, which was rarely mowed. *Plantago lanceolata* L. (Plantaginaceae), *Eragrostis pectinacea* (Michx.) Nees. (Poaceae), *Eleusine indica*, *Calyptocarpus vialis* Less. (Asteraceae) and *Indigofera spicata* Forssk (Fabaceae), were more common in areas nearer the paved roadways that were routinely mowed. Other weeds found in low



Table 9. 1. Percentage composition of weed flora in managed areas<sup>a</sup> on the paved roadway side of pineapple plantings.

Weed species	Transect Quadrat Section		
	I <sup>b</sup>	II <sup>c</sup>	III <sup>d</sup>
Bare ground	34.5	17.7	1.4
<i>Bidens pilosa</i>	21.1 (1)	19.9 (2)	1.4(7)
<i>Chloris gayana</i>	12.7 (2)	18.8 (3)	2.1 (6)
<i>Calyptocarpus vialis</i>	—	1.8(7)	12.1 (3)
<i>Digitaria insularis</i>	3.7 (5)	0.4	—
<i>Eleusine indica</i>	1.8	1.1	12.9 (2)
<i>Eragrostis pectinacea</i>	—	4.7 (4)	25.7 (1)
<i>Euphorbia</i> spp.	2.5(7)	3.6 (5)	—
<i>Indigofera spicata</i>	—	3.2(6)	7.1 (4)
<i>Rhynchelyrum repens</i>	7.6 (4)	—	—
<i>Plantago lanceolata</i>	1.1	21.3 (1)	25.7 (1)
<i>Panicum maximum</i>	3.6 (6)	1.8(7)	2.1 (6)
<i>Paspalum</i> spp.	2.5(7)	0.7	2.9 (5)
<i>Sorghum halepense</i>	5.1 (3)	1.1	—
<i>Sida</i> spp.	—	0.7	2.9 (5)

<sup>a</sup> Refers to roadside areas where weeds are routinely managed via mowing and herbicide applications (i.e., disturbed areas)

<sup>b</sup> 0 – 1 m from edge of pineapple planting

<sup>c</sup> 2 – 3 m from edge of pineapple planting

<sup>d</sup> 4 – 5 m from edge of pineapple planting

Figures within parentheses are the relative ranks of frequency of occurrence within the quadrat.

frequency included *Amaranthus* sp. L. (Amaranthaceae), *Cenchrus echinatus* L. (Poaceae), *Euphorbia* sp., *Lepidium densiflorum* Schrad. (Brassicaceae), *Ipomea* sp., *Oxalis* sp. L. (Oxalidaceae), *Ruellia prostrata* Poir (Acanthaceae), and *Sonchus* sp. L (Asteraceae).

During the initial surveys, two bands of different weed species were observed. Weedy plants near the roadway were predominantly *E. pectinacea*, *E. indica*, and *C. vialis* (Table 9.1, Quadrat III). These weeds received frequent mowing (i.e., every three months, County of Honolulu) and were shorter in height. Weeds nearer the pineapple border (Table 9.1, Quadrat I) were predominantly *B. pilosa*, *C. gayana* and *S. halepense*, which were rarely, if ever mowed. The weeds growing adjacent to the pineapple plantings were taller (> 1 m) and evidently suppressed or out competed other weeds. Weed species composition became more diverse as the distance from the pineapple border increased. *C. gayana*, *E. indica*, *R. repens* and *P. maximum* were the reported hosts of PPM found in this survey.

*Determination of Weed Diversity.* Of the 39 sites visited in August 2000, all of them had mowed bands of weeds and only 20 sites had unmowed bands. In January 2001, mowed bands were present in all 38 sites visited, but unmowed bands were only present in eight sites. During this survey period, plants at several sites looked unhealthy and dying following the application of herbicides. Manual removal of weeds from pineapple borders was also observed. The mowed bands of weeds were  $4.1 \pm 0.4$  m and  $3.9 \pm 0.3$  m wide in the August 2000 and January 2001 surveys, respectively (Table 9.2). In contrast, the unmowed bands were smaller than the mowed bands and measured

Table 9. 2. Widths of the unmowed and mowed weed bands and weed species density per survey site (1 m<sup>2</sup>).

Survey	Weed band	n	Width of weed band (m)	No. of weed species
				Density / m <sup>2</sup>
August 2000	Mowed	39	4.1 ± 0.4	6.8 ± 0.4
	Unmowed	20	2.5 ± 0.4	3.0 ± 0.4
January 2001	Mowed	38	3.9 ± 0.3	9.4 ± 0.4
	Unmowed	8	3.6 ± 0.4	3.5 ± 0.7

2.5 ± 0.4 m and 3.6 ± 0.4 m in August 2000 and January 2001, respectively ( $F_{1,90} = 3.91$ ,  $P = 0.051$ ). There was no statistical difference between the widths of the weed bands in either season ( $F_{1,90} = 0.89$ ,  $P = 0.349$ ) and there was no survey / weed band interaction ( $F_{1,90} = 2.10$ ,  $P = 0.151$ ). However, there was a significant interaction between the weed band (mowed or unmowed) ( $F_{1,101} = 88.85$ ,  $P < 0.0001$ ) and survey season relative to weed species density distribution ( $F_{1,101} = 4.55$ ,  $P = 0.035$ ) (Table 9.2). Weed density was highest in the mowed band in the January 2001 survey and lowest in the unmowed band in the August 2000 survey. Irrespective of the survey season, the mowed band had a higher weed species density than the unmowed band. Similarly, the January 2001 survey had a higher weed species density than the August 2000 survey ( $F_{1,101} = 9.09$ ,  $P = 0.003$ ).

Major weed species found during the surveys are presented in Table 9.3. The most common species in the mowed band were *Eragrostis pectinacea*, *Indigofera spicata*, *Plantago lanceolata*, *Calyptocarpus vialis*, *Cynodon dactylon* (L.) Pers., *Bidens pilosa* (L.), *Eleusine indica* and *Chloris gayana*. *Bidens pilosa*, *Chloris gayana* and *Digitaria insularis* were the most common weeds adjacent to pineapple in the unmowed band (Table 9.3). *Digitaria ciliaris* (Retz.) Koeler, *Euphorbia* spp., *Lepidium densiflorum*, *Chloris radiata* (L.) Pers., *Panicum maximum*, *Paspalum* spp. and *Cenchrus echinatus* were occasionally observed in the mowed band (Table 9.3). The weeds rarely recorded (from only a few sites) include *Amaranthus* sp., *Brachiaria mutica*, *Chloris virgata* Sw., *Chloris barbata* (L.) Sw., *Mimosa* sp., *Ipomea* spp., *Portulaca* sp. L. (Portulacaceae), *Rhynchelytrum repens*, *Ruellia prostrata*, *Sida* sp. L. (Malvaceae), *Sonchus* spp L. (Asteraceae) and *Sorghum halepense*.

Table 9. 3. Frequency of major weeds in disturbed plant communities adjacent to borders of pineapple plantings near paved roadways.

Weed species	Common name	August 2000		January 2001	
		Mowed n = 39 <sup>a</sup>	Unmowed n = 20	Mowed n = 38	Unmowed n = 8
<u>Asteraceae</u>					
<i>Bidens pilosa</i>	Spanish needle	22	14	32	5
<i>Calypocarpus vialis</i>		25	0	31	0
<u>Brassicaceae</u>					
<i>Lepidium densiflorum</i>	Peppergrass	4	0	7	0
<u>Euphorbiceae</u>					
<i>Euphorbia</i> spp.	Common spurge	3	1	20	1
<u>Fabaceae</u>					
<i>Indigofera spicata</i>		32	0	35	0
<u>Poaceae</u>					
<i>Cenchrus echinatus</i>	Common sand burr	5	4	7	0
<i>Chloris gayana</i>	Rhodes grass	16	11	25	5
<i>Chloris radiata</i>		5	0	10	0
<i>Cynodon dactylon</i>	Bermuda grass	24	1	17	0
<i>Digitaria ciliaris</i>		1	6	3	2
<i>D. insularis</i>	Sour grass	0	8	2	2
<i>Eleusine indica</i>	Wire grass	21	4	28	1
<i>Eragrostis pectinacea</i>	Carolina love grass	36	0	31	0
<i>Panicum maximum</i>	Guinea grass	2	2	9	3
<i>Paspalum</i> spp.		11	2	10	0
<i>Rhynchelytrum repens</i>	Natal grass	0	1	2	1
<u>Plantaginacea</u>					
<i>Plantago lanceolata</i>	Narrow leaved plantain	27	1	34	2

<sup>a</sup> n = total sites surveyed

Among the more common weeds recorded, *Chloris gayana* and *Eleusine indica* were the important PPM hosts and were infested with PPM during these surveys as well. Other plants that have been recorded as hosts of PPM, but were less common, were *Panicum maximum*, *Paspalum* spp., *Rhynchelytrum repens* and *Cenchrus echinatus*, which were free of PPM during these surveys. In each survey, infestation of weeds by PPM was recorded at three sites only. *Chloris gayana* was infested with mealybugs in both seasons, but *E. indica* was only infested in August 2000. Guinea grass, a commonly reported PPM hosts, was not only less frequently observed, but also was mealybug free during the surveys. Similarly, natal grass was also observed quite rarely and free from PPM except at one location.

*Mealybug density.* During the survey, *C. gayana*, *E. indica* and *R. repens* were found infested with PPM. Rhodes grass was the most common weed host with relatively high PPM densities (39 mealybugs per plant) (Table 9.4). No mealybugs were observed in guinea grass even when it was heavily foraged by the big headed ant, *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) and the adjacent pineapple crop was heavily infested with mealybugs.

*Mealybug Densities on Rhodes grass:* Of the sixty rhodes grass plants examined, only 41 were infested with mealybugs. The mean plant height of surveyed rhodes grass plants was  $34.4 \pm 3.8$  cm with a mean of  $8.5 \pm 1.9$  tillers per plant. There were about four vegetative tillers and almost one tiller each at booting, flowering, seed maturity, seed dispersal and head dying stages per plant (Table 9.5).

Correlation analysis showed that the total number of mealybugs per plant was significantly, but weakly, associated with plant characteristics such as plant height

Table 9. 4. PPM densities on various weed species adjacent to pineapple plantings.

Weed species	n	Mealybugs per plant (Mean $\pm$ SEM) <sup>a</sup>
<i>Chloris gayana</i>	45	39.3 $\pm$ 8.7
<i>Chloris radiata</i>	5	0.0 $\pm$ 0.0
<i>Digitaria insularis</i>	10	0.0 $\pm$ 0.0
<i>Eragrostis pectinacea.</i>	10	0.2 $\pm$ 0.2
<i>Rhynchelytrum repens</i>	5	14.6 $\pm$ 6.0
<i>Plantago lanceolata</i>	10	0.0 $\pm$ 0.0

<sup>a</sup> All PPM stages except crawlers.

Table 9. 5. Mean plant height and number of tillers per plant at various growth stages, and their correlation with total PPM and total tillers at seed dispersing stage per plant [ $r^2$  = correlation coefficient;  $P$  = associated probabilities,  $n = 41$ ].

Plant characteristics	Mean $\pm$ SEM	Total PPM		Seed dispersing tillers	
		$r^2$	$P$	$r^2$	$P$
Plant height (cm)	34.4 $\pm$ 3.8	0.295	< 0.001	0.310	< 0.001
<u>Number of tillers</u>					
Vegetative	4.1 $\pm$ 0.7	0.119	0.027	0.384	< 0.001
Booting	1.2 $\pm$ 0.3	0.397	< 0.001	0.613	< 0.001
Flowering	0.5 $\pm$ 0.1	0.061	0.120	0.199	0.003
Seed maturing	1.2 $\pm$ 0.4	0.250	0.001	0.638	< 0.001
Seed dispersing	0.9 $\pm$ 0.3	0.397	< 0.001	-	-
Dying head	0.9 $\pm$ 0.4	0.329	< 0.001	0.733	< 0.001



( $r^2 = 0.295$ ) and number of tillers, suggesting that larger plants have more mealybugs (Table 9.5). The association was even weaker when the number of vegetative tillers was examined ( $r^2 = 0.119$ ). A stronger, but still weak association was found with the number of reproductive tillers present at booting ( $r^2 = 0.397$ ) and in the seed dispersing stages ( $r^2 = 0.397$ ). A weak association was also found with the number of dying head tillers ( $r^2 = 0.329$ ) (Table 9.5).

Multiple regression analysis showed that number of tillers at the seed dispersing stage was the most useful variable to predict the mealybug density per plant. Once the number of seed dispersing tillers was added to the model, other variables (e.g., the numbers of vegetative, flowering, booting, maturity and dying tillers) were statistically non-significant due to multicollinearity, but the plant height remained statistically significant. The following equation explained about 43 % of the variability in the number of mealybugs per plant ( $F = 15.99$ ,  $df = 2,38$ ,  $P < 0.001$ ):

$$\text{Total PPM} = 0.61 + 5.36 (\text{seed dispersing tillers}) + 0.286 (\text{plant ht. in cm}) \quad [1]$$

*Parasitization.* A total of 615 adult mealybugs were collected over the period of four years from various weeds that were common around the pineapple borders (Table 9.6). All plants from which mealybugs were collected were inhabited by ants, mostly the big headed ant. None of these mealybugs produced any parasitoids.

The mealybugs collected from rhodes grass from all four locations within Oahu, Hawaii, were readily parasitized by *A. ananatis* in the laboratory (Table 9.7). None of the mealybugs in the control produced any mummies, indicating a lack of parasitization in the field.

Table 9. 6. Number of adult mealybugs collected and observed for parasitoid emergence from various weeds during the survey.

Year	Weed species	Total
1999	<i>Chloris gayana</i>	213
	<i>Eleusine indica</i>	1
	<i>Rhynchelytrum repens</i>	7
	<i>Chloris gayana</i>	46
2000	<i>Eleusine indica</i>	32
2001	<i>Chloris gayana</i>	240
2002	<i>Chloris gayana</i>	112
	Total	651

Table 9. 7. Number of mummies developed from mealybugs (MB) collected from *C. gayana* with and without exposure to *A. ananatis* in the laboratory.

Collection location	<i>A. ananatis</i>		Control	
	MB exposed	Mummified	MB exposed	Mummified
Manoa	12	3	12	0
Poamoho	25	7	25	0
Ewa	50	20	50	0
Kunia	25	4	25	0

## DISCUSSION

Weeds were common around pineapple plantings in both undisturbed and disturbed areas. Interestingly, weed diversity was low in the undisturbed areas with guinea grass being the predominant species. Although this weed is frequently mentioned as a PPM host in the scientific literature, none of the examined plants were infested with PPM despite their presence in adjacent pineapple plantings. The big headed ant commonly frequented this weed and may indirectly play an important role in the MWP. Weed species composition was more diverse in the disturbed areas adjacent to plantings, with the mowed areas being the most diverse. Although PPM is a polyphagous mealybug, able to infest more than 140 plants species, it was only found in high densities on a few plant species. The most common weed found in the unmowed bands was Spanish needle, which was not infested with PPM. Other common non-host weeds of PPM in the unmowed bands were sour grass and *Digitaria ciliaris*. The most common weed hosts of PPM included rhodes grass and wire grass, which were found in both the mowed and unmowed weed bands with the former species being more common. Only mature wire grass plants were infested. In contrast, all phenological stages of rhodes grass were infested with PPM.

The total PPM population in a weedy area would primarily depend on the density and distribution of host plant (e.g., rhodes grass) at the location and the mealybug density on the plant. Rhodes grass develops into large clumps that contain tillers at all growth stages. The number of tillers at the seed dispersing stage has a moderate correlation ( $r^2 = 0.384, 0.613, 0.199$  and  $0.638$ ) with the number of tillers at vegetative, booting, flowering

and seed maturity stages, respectively, suggesting it is a good indicator of the plant size as well as a weak estimator of the mealybug density on the plant ( $r^2 = 0.31$ ) (Table 9.5).

None of the mealybugs collected from the weeds produced parasitoids. These results suggest that the most common weeds found during these studies did not serve as a reservoir for *A. ananatis*. The failure to recover any natural enemy of PPM from weeds was not surprising because the PPM found on these weeds (e.g., rhodes grass, wire grass and natal grass) were always found at the crown region, mostly protected under the mud nests constructed by ants. Studies by Gonzalez-Hernandez *et al.* (1999) suggest that *A. ananatis* only parasitizes PPM individuals exposed on the aerial parts of their host plants.

Within the pineapple plantings, plastic mulch suppresses most of the weeds, so they are generally considered a minor problem within the crop. Because several weed species harbor *Dysmicoccus* mealybugs (Carter 1932a), weed management could play a significant role in MWP management. Although PPM that have fed on weeds do not transmit PMWaV (Carter 1951), migration of mealybugs from infested weeds could lead to MWP outbreaks in plantations where many asymptomatic pineapple plants may harbor PMWaV (Hu *et al.* 1996; Hu *et al.* 1997; Hughes and Samita 1998; Sether *et al.* 2001). Suppression of rhodes grass and other PPM weed hosts in disturbed areas with herbicides and the planting of flowering plants that produce nectar could potentially enhance biological control (by attracting and supplying food to mealybug parasitoids) while reducing a source of PPM around pineapple borders.

## REFERENCES

- Beardsley, J. W. 1959. On the taxonomy of pineapple mealybugs in Hawaii, with the description of previously unnamed species (Homoptera: Pseudococcidae).  
Proceedings of the Hawaiian Entomological Society 17: 29-37.
- Beardsley, J. W. 1965. Notes on the pineapple mealybug in Hawaii with the description of two new species (Homoptera: Pseudococcidae). Proceedings of the Hawaiian Entomological Society 19: 55-68.
- Ben Dov, Y. 1994. A Systematic Catalogue of the Mealybugs of the World (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with Data on Geographical Distribution, Host Plants, Biology and Economic Importance. Intercept, Andover, UK, 686 pp.
- Carter, W. 1932a. Pineapple mealybug, *Pseudococcus brevipes* (Ckll), occurring on pineapple plants. Ecology 13: 296-304.
- Carter, W. 1932b. Border plantings as guard rows in pineapple mealybug control. Journal of Economic Entomology 25: 1027-1031.
- Carter, W. 1933a. The pineapple mealybug *Pseudococcus brevipes* and wilt of pineapple. Phytopathology 23: 207-242.
- Carter, W. 1933b. The spotting of pineapple leaves caused by *Pseudococcus brevipes*, the pineapple mealybug. Phytopathology 23: 243-259.
- Carter, W. 1951. The feeding sequence of *Pseudococcus brevipes* (Ckll) in relation to mealybug wilt of pineapple in Hawaii. Phytopathology 41: 769-780.
- German, T. L., D. E. Ullman and U. B. Gunasinghe. 1992. Mealybug wilt of pineapple. Advances in Disease Vector Research 9: 241-259.

- Gonzalez-Hernandez, H. 1995. The Status of Biological Control of Pineapple Mealybugs in Hawaii. Ph.D Dissertation, University of Hawaii at Manoa, Honolulu, Hawaii.
- Hu, J. S., D. M. Sether and D. E. Ullman. 1996. Detection of pineapple Closterovirus in pineapple plants and mealybugs using monoclonal antibodies. *Plant Pathology* 45: 829-836.
- Hu, J. S., D. M. Sether, X. P. Liu, M. Wang, F. Zee and D. E. Ullman. 1997. Use of a tissue blotting immunoassay to examine the distribution of pineapple closterovirus in Hawaii. *Plant Disease* 81: 1150-1154.
- Hughes, G. and S. Samita. 1998. Analysis of patterns of pineapple mealybug wilt disease in Sri Lanka. *Plant Disease* 82: 885-890.
- Illingworth, J. F. 1931. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. *Journal of Economic Entomology* 24: 877-889.
- Jahn, G. C. and J. W. Beardsley. 2000. Interactions of ants (Hymenoptera: Formicidae) and mealybugs (Homoptera: Pseudococcidae) on pineapple. *Proceedings of the Hawaiian Entomological Society* 34: 181-185.
- Noyes, J. S. and M. Hayat. 1994. Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae). Natural History Museum/CAB International, London, 554 pp.
- Rohrbach, K. G. and W. J. Apt. 1986. Nematode and disease problems of pineapple. *Plant Disease* 70: 81-87.
- Rohrbach, K. G., J. W. Beardsley, T. L. German, N. J. Reimer and W. G. Sanford. 1988. Mealybug wilt, mealybugs, and ants of pineapple. *Plant Disease* 72: 558-565.

- Sether, D. M., D. E. Ullman and J. S. Hu. 1998. Transmission of pineapple mealybug wilt-associated virus by two species of mealybug (*Dysmicoccus* spp.).  
Phytopathology 88: 1224-1230.
- Sether, D. M., A. V. Karasev, C. Okumura, C. Arakawa, F. Zee, M. M. Kislán, J. L. Busto and J. S. Hu. 2001. Differentiation, distribution, and elimination of two different Pineapple mealybug wilt-associated viruses found in pineapple. Plant Disease 85: 856-864.
- Williams, D. J. and G. W. Watson. 1988. The Scale Insects of Tropical South Pacific Region. CAB International, Wallingford, 260 pp.
- Williams, D. J. and M. C. G. D. Willink. 1992. Mealybugs of Central and South America. CAB International, Wallingford, 635 pp.



## X. SUMMARY AND RECOMMENDATIONS

Studies were conducted on the pink pineapple mealybug (PPM), *Dysmicoccus brevipes* (Cockerell), and its associated encyrtid natural enemy, *Anagyrus ananatis* Gahan. Rearing methodologies for PPM were refined to permit maximum utilization of squash fruit for producing mealybug hosts for *A. ananatis* parasitization. Several biological parameters (e.g., developmental rates, tolerance to cool temperatures, influence of host size on parasitoid fecundity) of *A. ananatis* were studied that were useful for developing mass production and augmentation techniques. The potential of reducing the disruptive effects of ants on PPM biological control was evaluated under laboratory conditions by establishing high parasitoid to ant ratios to limit the ants ability to protect PPM. Lastly, weed flora neighboring pineapple plantings was surveyed to determine the role of common weeds as a source of PPM infestation for pineapple and as a reservoir for its natural enemies.

### **PPM Mass Production**

Kobocho squash, *Cucurbita moschata* (Duch. Ex Lam) Duch ex Poir, was more appropriate than butternut squash for mass rearing mature adult PPM suitable for parasitoid reproduction. PPM produced honeydew that accumulated on the squash surface, entrapped immatures, and limited PPM production. Each mature adult PPM could produce about 0.4 mg of honeydew daily. Mealybug wax, exuviae and crawlers were found stuck in the residual honeydew if not periodically removed from the squash surface. Use of coarse vermiculite particles (> 2.36 mm diameter) absorbed the honeydew, and weekly stirring of the contaminated particles dispersed the PPM-produced

honeydew, wax, and molted casts, which gave the squash surface a clean and dry appearance in contrast to fruit upon which the honeydew was permitted to accumulate. No obvious impediments to PPM reproduction were observed with the use of vermiculite. However, the microenvironment of the rearing chamber must be well ventilated, and the rearing containers need to be permeable to facilitate moisture loss. The number of mealybugs produced for parasitoid propagation ( $> 0.6$  mg) was highest (ca. 2,200 individuals/kg squash) when 0.5 g (= 200-300 individuals) of mature adults were used for inoculation. The time required for the mealybugs to mature was about 7 to 8 weeks at  $23 \pm 1^\circ\text{C}$ .

#### **Biological Parameters of *Anagyrus ananatis***

*Influence of nutrition on adult longevity.* Separate provision of honey and water was beneficial for the mass production of *A. ananatis* in the laboratory. However, provision of honey following adult emergence was not beneficial to the parasitoid if not continuously supplied throughout its life. Thus, additional carbohydrate sources must be located by *A. ananatis* following an augmentative release in order to extend its longevity in the field.

*Influence of photophase on parasitoid activity.* Parasitoid emergence occurred in the early morning hours and oviposition occurred during photophase only. Early morning field releases would coincide with natural parasitoid eclosion, providing an entire day for searching. Late evening releases would potentially give individuals time to acclimate themselves to the crop environment before beginning to search the next morning.

*Effect of host PPM size on parasitoid size.* The use of PPM adults as hosts for producing *A. ananatis* would produce larger females with female-biased sex ratios than if younger PPM stages were used. PPM reared on squash should be harvested and sized, using sieves, to isolate useful stages. Only PPM individuals larger than 0.6 mg in weight should be used for rearing *A. ananatis*. The size of a PPM mummy was a good predictor of the sex and eventual adult body size of the *A. ananatis* individual contained within, and may be used as an accurate indicator of parasitoid quality in a mass production system. Female *A. ananatis* body size was a major contributing factor in determining the reproductive ability of parasitoids reared from PPM hosts that were denied access their plant host (i.e., squash) following parasitization. It is important to produce adequately large-sized female parasitoids to ensure normal fecundity.

*Temperature requirements for development.* *Anagyrus ananatais* has a lower developmental threshold temperature ( $T_0$ ) around 12.65°C and higher threshold close to 31°C. Total accumulated temperature (K) was determined to be 265 Degree Days (DD) for males and 275 DD for females above the  $T_0$ . Few individuals completed their developmental cycle at 14.6°C, which was about 2°C above the  $T_0$ . Temperatures from 19 to 29°C are optimal for rearing *A. ananatis*, with the most optimal temperature being around 24°C. The time of adult *A. ananatis* emergence from the initiation of the mummy stage may be predicted based on the incubation temperature and the developmental stage. The rearing temperature can be manipulated within the optimal range of temperature to shorten or lengthen the developmental time of the parasitoid. *A. ananatis* completed its egg to larval development in about eight days, but took much longer (ca. 14 days) to

complete pupal development at 23.5°C. Although *A. ananatis* is a koinobiont, the host mealybug was killed within a few (6-8) days after parasitization.

*Cool temperature storage.* *Anagyrus ananatis* has a wide range of variability in terms of cool temperature tolerance. The effects of cool temperature storage were time dependent, with longer storage periods inducing higher mortality and lowered fecundity. Immatures within mummies could be stored up to 6 weeks at 14.8°C without affecting their survival and longevity, but fecundity was severely affected. Preconditioning immatures at a cool temperature above the  $T_o$  (14.8°C) for one week before exposing them to a temperature below the  $T_o$  (10.1°C) reduced the deleterious effect of cool temperature and improved survival rate and fecundity compared to those without preconditioning. Preconditioning of immatures at 14.8°C for one week and then storage at 10.1°C resulted in a relatively higher emergence rate as well as a normal fecundity rate and may be employed for storage as well as transportation of the mummies. Prepupal to early pupal stages can only be stored for brief periods (< 2 weeks) at 10.1°C (below the  $T_o$ ) because prolonged exposure is lethal.

Although parasitoid emergence and longevity are important, the most important traits for biological control are reproductive ability coupled with host searching ability. These results suggest that survival and longevity may give faulty estimates of the fitness of stored parasitoids. The effect of cold temperature appears to be more detrimental to male parasitoids than females.

### **Overcoming the Impact of Ants**

The big headed ant (BHA), *Pheidole megacephala* F., transported PPM. BHA effectively reduced PPM parasitization by *A. ananatis* to almost half of that recorded on

mealybugs in the absence of BHA. Increasing *A. ananatis* densities in the presence of BHA increased PPM parasitization rates and suggested that augmentative field releases of *A. ananatis* may aid in the suppression of PPM in the presence of BHA. The mean reduction in the PPM parasitization rate due to ant interference in this study ranged from 18 to 56% compared to the control.

### **Survey of Weeds Around Pineapple Plantings**

Guinea grass, *Panicum maximum*, which is frequently mentioned as a PPM host in the scientific literature, was the predominant weed species in undisturbed areas bordering pineapple plantings, but none of the examined plants were infested with PPM. The most common weed hosts of PPM included rhodes grass, *Chloris gayana*, and wire grass, *Eleusine indica*, which were found in both mowed and unmowed weed bands within disturbed areas with the former species being more common. Only mature wire grass plants were PPM infested. In contrast, all phenological stages of rhodes grass were infested with PPM. None of the mealybugs collected from the examined weeds produced parasitoids of any kind. These results suggest that the most common weeds found during these studies did not serve as a reservoir for *A. ananatis*. Suppression of rhodes grass and other PPM weed hosts in disturbed areas with herbicides and the planting of flowering plants that produce nectar could potentially enhance biological control while reducing a source of PPM around pineapple borders.

### **Future Research Recommendations**

*Anagyrus ananatis* can be mass produced following the methods described above. Laboratory studies on overcoming the disruptive effects of BHA on PPM biological control were promising, but the real effectiveness of parasitoid releases can only be

determined under field conditions. Further studies on *A. ananatis* augmentation should be conducted to evaluate whether inoculative releases are adequate or if inundative field releases are required to achieve the desired level of PPM suppression (i.e., to inhibit mealybug wilt of pineapple). Further studies need to be conducted to develop effective parasitoid field-delivery methods, timing of releases, and field release rates.

Continuous storage of *A. ananatis* immature stages at a temperature above, but close to, the  $T_o$  did not affect parasitoid survival. However, it did result in the loss of reproductive ability compared to the control as well as those individuals that were preconditioned at the same temperature and then stored below  $T_o$ . Most insects are stored and transported at cool temperatures. Further investigations to determine the effect of such cool temperatures (above and/or below  $T_o$ ) on the developmental physiology of an insect should help in the understanding of the overall fitness of biological control agents mass-produced for augmentative biological control.

The optimal temperature for *A. ananatis* development was determined to range from 19 to 29°C. Whether *A. ananatis* individuals developed at various temperatures within the estimated optimal temperature would differ in their reproductive ability needs to be examined further.