Mass Production of Beneficial Organisms
Invertebrates and Entomopathogens

Edited by
Juan A. Morales-Ramos
USDA-ARS
National Biological Control Laboratory
Stoneville, MS, USA

M. Guadalupe Rojas
USDA-ARS
National Biological Control Laboratory
Stoneville, MS, USA

David I. Shapiro-Ilan
USDA-ARS, SAA
SE Fruit and Tree Nut Research Unit
Byron, GA, USA
Section I

1. Introduction

Norman C. Leppla, Juan A. Morales-Ramos, David I. Shapiro-Ilan and M. Guadalupe Rojas

1.1. Challenges of Mass Producing Beneficial Organisms 3
1.2. Challenges of Arthropod Mass Production for Biological Control 7
1.3. Challenges of Mass Producing Pathogens for Biological Control 9
1.4. Challenges of Mass Rearing Invertebrates for their Products and Ecological Services 11

References 12

2. Production of Coleopteran Predators

Eric W. Riddick and Hongyin Chen

2.1. Introduction 17

2.1.1. Aims of this Chapter 17
2.1.2. Predatory Beetles in Culture 18
2.1.3. Overview of the Content 18

2.2. Foods and Production of Predators 22

2.2.1. Feeding Preferences and Natural Prey 22
2.2.2. Feeding on Factitious Foods and Plant Products 23
2.2.3. Feeding on Artificial Diets 26

2.3. Rearing Density and Production 34

2.3.1. Crowding 34
2.3.2. Cannibalism 35
2.3.3. Design of Oviposition Substrates and Rearing Enclosures 36
2.3.4. Rearing Scale 37

2.4. Temperature and Production 38

2.4.1. Optimizing Temperature for Rearing 38
2.4.2. Reducing Temperature for Cold Storage 39

2.5. Quality Control and Production 42

2.5.1. Safeguards against Unwanted Pathogens and Parasites 42
2.5.2. Preventing Colony Deterioration 42
2.5.3. In-Shipment, Post Shipment, and Prerelease Assessments 43
2.6. Conclusions and Recommendations 44
  2.6.1. Synthesis 44
  2.6.2. Future Research 45
Acknowledgments 45
References 46
Further Reading 55

3. Production of Heteropteran Predators
*Patrick De Clercq, Thomas A. Coudron and Eric W. Riddick*

3.1. Introduction 57
3.2. Foods 61
  3.2.1. Natural Prey 61
  3.2.2. Factitious Prey 63
  3.2.3. Artificial Diets 67
3.3. Plant Materials and Alternatives 79
  3.3.1. Plant Substrates 79
  3.3.2. Artificial Substrates 81
3.4. Crowding and Cannibalism 84
3.5. Microorganisms 85
3.6. Breeding and Colony Maintenance 86
3.7. Mass-Rearing Systems 88
3.8. Conclusions 89
Acknowledgments 90
References 90
Further Reading 100

4. Production of Dipteran Parasitoids
*Maria Luisa Dindo and Simon Grenier*

4.1. Introduction 101
4.2. Dipteran Parasitoids as Biocontrol Agents 102
  4.2.1. Tachinidae 102
  4.2.2. Other Dipteran Parasitoids 107
  4.2.3. Side Effects 108
4.3. Aspects of Dipteran Parasitoid Biology of Special Interest for Production 109
  4.3.1. Host Range 109
  4.3.2. Oviposition Strategies 110
  4.3.3. Host–Parasitoid Interactions 112
4.4. Production Techniques 114
  4.4.1. In Vivo Production 114
  4.4.2. In Vitro Production 118
  4.4.3. Adult Maintenance 123
  4.4.4. Quality Control 126
  4.4.5. Storage and Shipment Procedures 128
4.5. Perspectives and Concluding Remarks 129
References 131
5. Mass Rearing *Bemisia* Parasitoids for Support of Classical and Augmentative Biological Control Programs

*John A. Goolsby, Matthew A. Ciomperlik, Gregory S. Simmons, Charles J. Pickett, Juli A. Gould and Kim A. Hoelmer*

5.1. Introduction 145
5.2. Laboratory Culture 148
5.3. Outdoor Field Cage Production 150
5.4. Large-Scale Greenhouse-Based System 152
5.5. Conclusion 160
5.6. USDA Disclaimer 161
References 162
Further Reading 162

6. Mass Rearing of the Stem-Galling Wasp *Tetramesa romana*, a Biological Control Agent of the Invasive Weed *Arundo donax*


6.1. Introduction 163
   6.1.1. Critical Needs in a Mass Rearing Program for Biological Weed Control 164
6.1.2. Mass Rearing of Weed Biological Control Agents: Past Successes 166
6.1.3. The Critical Need for Mass Rearing Programs for Exotic Invasive Environmental Weeds 169
6.1.4. *Arundo donax* as an Invasive Weed 170
6.2. Biological, Ecological, and Behavioral Information about the Arundo Wasp 171
   6.2.1. Discovery and Characterization 171
   6.2.2. Considerations for Mass Rearing of the Arundo Wasp 174
6.3. A Plant-Based Mass Rearing System for the Arundo Wasp 177
   6.3.1. Description of the Mass Rearing System 177
   6.3.2. Output of the Mass Rearing System 181
6.4. Challenges Encountered and Addressed in the Development of Mass Rearing 182
   6.4.1. Challenges to Efficient Mass Rearing 182
   6.4.2. Key Challenges Addressed in Mass Rearing 183
6.5. Use of Mass Reared Arundo Wasps for Biological Control of Arundo 185
   6.5.1. Use of Cold Inebriation to Prolong Wasp Life 185
   6.5.2. Packaging of Wasps for Field Release 185
   6.5.3. Status of the Arundo Wasp Mass Release Program 186
6.6. Investigations into an Artificial Diet for Rearing of the Arundo Wasp 186
   6.6.1. Nutritional Needs and Feeding Constraints 186
   6.6.2. Novel Feeding Modality of the Arundo Wasp Larva 187
8.3. Case Studies

8.3.1. Complete Cohort Life Table of the Southern Green Stink Bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) 250

8.3.2. Abridged Cohort Life Table for the CBB, *Hypothenemus hampei*, at Different Temperatures Using an Artificial Diet 256

8.3.3. Life Tables for *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylidae) Ectoparasitoids of *Hypothenemus hampei*, Using an Artificial Diet 261

8.3.4. Comparison of Demographic Parameters of *Perillus bioculatus* Feeding on Factitious Prey (*Anthonomus grandis*) Larvae and Natural Prey (*Leptinotarsa decemlineata*) Eggs 267

8.4. Concluding Remarks

References 273


*Norman C. Leppla*

9.1. Introduction 277

9.2. Quality Assurance in the Marketplace 280

9.3. Customer Involvement in Quality Assurance 282

9.4. Building a Complete Quality Assurance System 283

9.4.1. Management 283

9.4.2. Methods Development 285

9.4.3. Materials 285

9.4.4. Production 285

9.4.5. Research 286

9.4.6. Utilization 286

9.4.7. Personnel 287

9.4.8. Quality Control 288

9.5. Quality Assessments of Mass-Reared Natural Enemies 288

9.6. Quality Assurance and Control Data Acquisition and Analysis 294

9.7. Quality Assurance System Review 295


9.9. Conclusion 303

Acknowledgments 305

References 305

Section II

10. Production of Entomopathogenic Nematodes

*David I. Shapiro-Ilan, Richou Han and Xuehong Qiu*

10.1. Introduction 321

10.2. In Vivo Production 326

10.2.1. Basic Method 326
10.2.2. Factors Affecting Efficiency 327
10.2.3. Recent Advances and Future Directions 329
10.3. In Vitro Production: Solid Culture 331
10.3.1. Basic Method 331
10.3.2. Factors Affecting Efficiency 333
10.3.3. Recent Advances and Future Directions 334
10.4. In Vitro Production: Liquid Culture 335
10.4.1. Basic Method 335
10.4.2. Factors Affecting Efficiency 337
10.4.3. Recent Advances and Future Directions 338
10.5. Analysis and Conclusion 339
10.5.1. Comparison of Production Methods 339
10.5.2. Strain Selection, Improvement and Stability 341
10.5.3. Conclusion 345
References 346

11. Mass Production of Entomopathogenic Fungi: State of the Art
Stefan T. Jaronski

11.1. Introduction 357
11.2. Production Methods for the Important Insect Pathogenic Fungi
11.2.1. Lagenidium giganteum (Schenk) 360
11.2.2. Leptolegnia chapmani 361
11.2.3. Coelomomyces spp. Keilin 361
11.2.4. Entomophthorales 361
11.2.5. Microsporidia 365
11.2.6. Ascomycete Hypocreales 366
11.3. Process and Quality Control in Mass Production 391
11.4. Current Knowledge about Effect of Cultural Conditions on Propagule Attributes
11.4.1. Age of Conidia 394
11.4.2. Conidia Produced under Certain Nutrient Conditions or under Osmotic Stress 395
11.4.3. Conidia Produced after Photoirradiation 397
11.5. The Challenge in Mass Production of Entomopathogenic Fungi 397
References 400

12. Commercial Production of Entomopathogenic Bacteria
Terry L. Couch and Juan Luis Jurat-Fuentes

12.1. Biology of Commercial Bacterial Entomopathogens 415
12.2. Biology of Commercial Bacterial Entomopathogens 417
12.3. Pathogenesis and Pest Control Impact 419
12.4. Culture Selection and Maintenance 421
12.5. Inoculum Preparation for Entomopathogenic Bacteria 422
12.6. Fermentation Medium Selection 423
12.7. Recovery and Concentration Steps 426
  12.7.1. Recovery 426
12.8. Formulation Selection 427
12.9. Formulation Standardization 430
12.10. Quality Assurance Methods 430
12.11. Conclusion 431
References 431
Further Reading 435

13. Production of Entomopathogenic Viruses
Steve Reid, Leslie Chan and Monique M. van Oers

13.1. Introduction 437
  13.1.1. Entomopathogenic Viruses 438
  13.1.2. Baculoviruses 438
13.2. In Vivo Production of Baculovirus-Based Biopesticides 442
13.3. In Vitro Production: Current Status 444
  13.3.1. Cell Lines Available 444
  13.3.2. Virus Isolates Available 445
  13.3.3. Low-Cost Media 447
  13.3.4. Current Status of Bioreactor-Based Production: HearNPV as a Case Study 449
13.4. Limitations to Bioreactor Production of Baculovirus-Based Pesticides 454
  13.4.1. Lack of a Chemically Defined Media 454
  13.4.2. Low Budded Virus Titers 455
  13.4.3. ODVs Produced in Cell Culture May Have a Lower Speed of Kill 456
  13.4.4. Viral Genome Instability during In Vitro Passaging 457
  13.4.5. Complications with High-Density Cell Culture 461
13.5. Future Research Directions for Bioreactor Production of Baculovirus-Based Pesticides 462
  13.5.1. Chemically Defined Media for Insect Cell Culture 462
  13.5.2. Genomics/Transcriptomics of Insect Cell Lines 463
  13.5.3. Metabolomics of Insect Cell Lines 464
  13.5.4. Genetically Modified Cell Lines/Viruses 464
  13.5.5. Future Potential 466
13.6. Conclusions 467
Acknowledgments 470
References 470

14. Formulations of Entomopathogens as Bioinsecticides
Robert Behle and Tim Birthisel

14.1. Introduction 483
  14.1.1. Goals and Benefits of Formulations 484
  14.1.2. Challenges of Microbial Pesticides 486
14.2. Biological Considerations 488
  14.2.1. Biological Attributes for the Microbe 488
  14.2.2. Potential Hazards 494
14.3. Physical Considerations 497
  14.3.1. Cost 497
  14.3.2. Formulation Form 498
  14.3.3. Ingredients 500
  14.3.4. Processing 502
  14.3.5. Mixing, Handling, and Packaging 506
  14.3.6. Consumer Aesthetics 506
  14.3.7. Application 507
14.4. Additional Considerations on Formulation 508
  14.4.1. Sources of Technologies 508
  14.4.2. Legal Requirements 508
  14.4.3. Current Effective Formulations 509
  14.4.4. Unique Applications 509
14.5. Conclusions and Future of Biopesticide Formulations 510
References 511

15. Mass Production of Entomopathogens in Less Industrialized Countries
   David Grzywacz, David Moore and R.J. Rabindra

15.1. Introduction 519
15.2. Issues and Opportunities for Entomopathogen Uptake in Less Industrialized Countries 520
15.3. Practical Constraints for Entomopathogen Uptake in Developing Countries 520
15.4. Production of Entomopathogens in Less Industrialized Countries 521
15.5. Production of Entomopathogenic Fungi 522
  15.5.1. The LUBILOSA System 524
  15.5.2. The Caroni System 525
15.6. Additional Examples from Other Countries 527
  15.6.1. China 527
  15.6.2. Brazil 527
  15.6.3. Cuba 527
  15.6.4. Honduras 528
  15.6.5. Kenya and South Africa 528
15.7. Other Systems 529
15.8. Mass Production of Baculoviruses 529
  15.8.1. Country Case Studies 536
15.9. Other Production Systems 539
15.10. Generic Production Issues 541
  15.10.1. Product Quality 541
  15.10.2. Product Quantity 542
  15.10.3. Safety 545
  15.10.4. Economics 546
15.11. Requirements for Establishing Biopesticide Industries in LICs

15.11.1. Research and Information 546
15.11.2. Registration and Regulation in LIC 551
15.11.3. Responsibility 552
15.11.4. Future 552

Acknowledgments 553
References 553

Section III

16. Insect Protein as a Partial Replacement for Fishmeal in the Diets of Juvenile Fish and Crustaceans

Eric W. Riddick

16.1. Introduction 565
16.1.1. The Need for Alternatives to Fishmeal 565
16.1.2. Aims of this Chapter 566
16.1.3. Overview of the Content 566

16.2. Model Insects and Potential as Feed for Fish 566
16.2.1. Oriental Silkworm Moth and Relatives 566
16.2.2. House Fly and Black Soldier Fly 568
16.2.3. Yellow Mealworm and Superworm 571

16.3. Challenges and Opportunities to Expansion of Market for Insects as Feed 572
16.3.1. Artificial Diets 572
16.3.2. Scale-Up of Production 574

16.4. Quality Control and Production 575
16.4.1. Maintenance of Long-Term Insect Colonies 575
16.4.2. Colony Hygiene and Preventing Disease Transmission 575

16.5. Conclusions and Recommendations 576
16.5.1. Synthesis 576
16.5.2. Future Research 578

Acknowledgments 579
References 579

17. Insects as Food for Insectivores

Mark D. Finke and Dennis Oonincx

17.1. Introduction 583
17.2. Nutrient Content of Insects 584
17.2.1. Protein and Amino Acids 584
17.2.2. Fats and Fatty Acids 586
17.2.3. Carbohydrates 589
17.2.4. Fiber and Chitin 589
17.2.5. Minerals 590
17.2.6. Vitamins and Carotenoids 592
17.3. Effects of Insect Size/Life Stage on Nutrient Composition

17.4. Effects of Insect Diet on Insect Nutrient Composition

17.5. Effects of Environment on Insect Composition
17.5.1. Temperature
17.5.2. Humidity
17.5.3. Photoperiod

17.6. Nutrient Requirements of Insectivores, Including Diet Availability
17.6.1. Availability and Digestibility

17.7. Enhancing the Nutrient Composition of Insects as Food for Insectivores
17.7.1. Gut Loading
17.7.2. Dusting

17.8. Other Considerations
17.8.1. Pathogens/Parasites
17.8.2. Toxins

17.9. Conclusions

References

18. Insects for Human Consumption

*Marianne Shockley and Aaron T. Dossey*

18.1. Introduction

18.2. Historic and Cultural Precedents for Insects as Food
18.2.1. History of Human Insect Consumption
18.2.2. Overview of Insect-Eating Cultures in Modern Times
18.2.3. Cultural Acceptance of Edible Insects
18.2.4. Social Change, Food Choice, and Perceptions

18.3. Nutritional and Human Health Value of Insects
18.3.1. Animal- versus Plant-Based Food and Protein
18.3.2. Nutrient Content of Insects
18.3.3. Global Malnutrition and How Insects Can Help
18.3.4. Insects in Medicine and Drug Discovery

18.4. Insects as a Sustainable Source of Human Food
18.4.1. Environmental Footprint of Insects versus Vertebrate Livestock
18.4.2. Insects as Animal Feed
18.4.3. Considerations for Insect-Based Food Production, Processing, and Safety

18.5. Current Examples of Mass-Produced Insects with Potential as Human Food
18.5.1. Orthoptera
18.5.2. Diptera
18.5.3. Coleoptera
18.5.4. Lepidoptera

18.6. Potential Products and Byproducts from Mass-Produced Food or Feed Insects
18.6.1. Alternative “Meats”
19. Production of Solitary Bees for Pollination in the United States
Stephen S. Peterson and Derek R. Artz

19.1. Introduction 653
19.2. The Alfalfa Leafcutting Bee 654
19.3. The Alkali Bee 662
19.4. The Blue Orchard Bee 664
19.6. Other Solitary Bees of Interest for Pollination 673
19.7. Concluding Remarks 674
Acknowledgments 675
References 675

Christopher N. Lowe, Kevin R. Butt and Rhonda L. Sherman

20.1. Introduction 683
20.1.1. Ecological Groupings 684
20.1.2. Selection of Species 685
20.1.3. Cultivation Techniques 685
20.2. Current Applications 688
20.2.1. As a Protein Source 688
20.2.2. In Organic Waste Management 689
20.2.3. As Fishing Bait 691
20.2.4. In Soil Restoration 692
20.2.5. In Agro-Ecosystems 694
20.2.6. In Laboratory Experimentation 696
20.2.7. In Ecotoxicology 699
20.3. The Future for Mass Earthworm Culture 700
References 703
Further Reading 709

Index 711