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CRC Handbook of Natural Pesticides

Volume V

Edited by

Carlo M. Ignoffo

Part A

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CRC Handbook of Natural Pesticides

Volume V
Microbial Insecticides
Part A
Entomogenous Protozoa and Fungi

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CRC Series in Naturally Occurring Pesticides

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

First published 1988 by CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Reissued 2018 by CRC Press

ISBN 13: 978-1-138-59702-0 (hbk)
ISBN 13: 978-0-429-48718-7 (ebk)

Visit the Taylor & Francis Web site at http://www.taylorandfrancis.com and the
CRC Press Web site at http://www.crcpress.com

ERRATA

CRC Handbook of Natural Pesticides
Volume V: Microbial Insecticides, Part A

Headings on certain pages in the book are incorrect due to a printing error. The headings should appear as follows:

page 19 ***Ascogregarina culicis*** **(Ross, 1898)**
page 31 ***Nosema fumiferanae*** **(Thomson, 1955)**
page 37 ***Nosema pyrausta*** **(Paillot, 1927)**
page 41 ***Vairimorpha necatrix*** **(Kramer, 1965)**
page 44 ***Vavraia culicis*** **(Weiser, 1947)**

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CRC Handbook Series in Naturally Occurring Pesticides

INTRODUCTION

The United States has been blessed with high quality, dependable supplies of low cost food and fiber, but few people are aware of the never-ending battle that makes this possible. There are at present approximately 1,100,000 species of animals, many of them very simple forms, and 350,000 species of plants that currently inhabit the planet earth. In the U.S. there are an estimated 10,000 species of insects and related acarinids which at sometime or other cause significant agricultural damage. Of these, about 200 species are serious pests which require control or suppression every year. World-wide, the total number of insect pests is about ten times greater. The annual losses of crops, livestock, agricultural products, and forests caused by insect pests in the U.S. have been estimated to aggregate about 12% of the total crop production and to represent a value of about $4 billion (1984 dollars). On a world-wide basis, the insect pests annually damage or destroy about 15% of total potential crop production, with a value of more than $35 billion, enough food to feed more than the population of a country like India. Thus, both the losses caused by pests and the costs of their control are considerably high. Insect control is a complex problem for there are more than 200 insects that are or have been subsisting on our main crops, livestock, forests, and aquatic resources. Today, in the U.S., conventional insecticides are needed to control more than half of the insect problems affecting agriculture and public health. If the use of pesticides were to be completely banned, crop losses would soar and food prices would also increase dramatically.

About 1 billion pounds of pesticides are used annually in the U.S. for pest control. The benefits of pesticides have been estimated at about $4/$1 cost. In other words, chemical pest control in U.S. crop production costs an estimated $2.2 billion and yields a gross return of $8.7 billion annually.

Another contributing factor for increased crop production is the effective control of weeds, nematodes, and plant diseases. Crop losses due to unwanted weed species are very high. Of the total losses caused by pests, weeds alone count for about 10% of the agricultural production losses valued at more than $12 billion annually. Farmers spend more than $6.2 billion each year to control weeds. Today, nearly all major crops grown in the U.S. are treated with herbicides. As in insect pest and weed control programs, several chemicals are used in the disease programs. Chemical compounds (e.g., fungicides, bactericides, nematicides, and viracides) that are toxic to pathogens are used for controlling plant diseases. Several million dollars are spent annually by American farmers to control the diseases of major crops such as cotton and soybeans.

Another aspect for improved crop efficiency and production is the use of plant growth regulators. These chemicals that regulate the growth and development of plants are used by farmers in the U.S. on a modest scale. The annual sale of growth regulators is about $130 million. The plant growth regulator market is made up of two distinct entities — growth regulators and harvest aids. Growth regulators are used to increase crop yield or quality. Harvest aids are used at the end of the crop cycle. For instance, harvest aids defoliate cotton before picking or desiccate potatoes before digging.

The use of modern pesticides has accounted for astonishing gains in agricultural production as the pesticides have reduced the hidden toll exacted by the aggregate attack of insect pests, weeds, and diseases, and also improved the health of humans and livestock as they control parasites and other microorganisms. However, the same chemicals have allegedly posed some serious problems to health and environmental safety, because of their high toxicity and severe persistence, and have become a grave public concern in the last 2 decades. Since the general public is very much concerned about their hazards, the U.S. Environmental

Protection Agency enforced strong regulations for use, application, and handling of the pesticides. Moreover, such toxic pesticides as DDT, 2,4,5-T and toxaphene were either completely banned or approved for limited use. They were, however, replaced with less dangerous chemicals for insect control. Newer approaches for pest control are continuously sought, and several of them look very promising.

According to a recent study by the National Academy of Sciences, pesticides of several kinds will be widely used in the foreseeable future. However, newer selective and biodegradable compounds must replace older highly toxic persistent chemicals. The pest control methods that are being tested or used on different insects and weeds include: (1) use of natural predators, parasites, and pathogens, (2) breeding of resistant varieties of species, (3) genetic sterilization techniques, (4) use of mating and feeding attractants, (5) use of traps, (6) development of hormones to interfere with life cycles, (7) improvement of cultural practices, and (8) development of better biodegradable insecticides and growth regulators that will effectively combat the target species without doing damage to beneficial insects, wildlife, or man. Many leads are now available, such as the hormone mimics of the insect juvenile and molting hormones. Synthetic pyretheroids are now replacing the conventional insecticides. These insecticides, which are a synthesized version of the extract of the pyrethrum flower, are much more attractive biologically than the traditional insecticides. Thus, the application rates are much lower in some cases, one tenth the rates of more traditional insecticides such as organophosphorus pesticides. The pyrethroids are found to be very specific for killing insects and apparently exhibit no negative effects on plants, livestock, or humans. The use of these compounds is now widely accepted for use on cotton, field corn, soybean, and vegetable crops.

For the long term, integrated pest management (IPM) will have tremendous impact on pest control for crop improvement and efficiency. Under this concept, all types of pest control — cultural, chemical, inbred, and biological — are integrated to control all types of pests and weeds. The chemical control includes all of the traditional pesticides. Cultural controls consist of cultivation, crop rotation, optimum planting dates, and sanitation. Inbred plant resistance involves the use of varieties and hybrids that are resistant to certain pests. Finally, the biological control involves encouraging natural predators, parasites, and microbials. Under this system, pest-detection scouts measure pest populations and determine the best time for applying pesticides. If properly practiced, IPM could reduce pesticide use up to 75% on some crops.

The naturally occurring pesticides appear to have a prominent role for the development of future commercial pesticides not only for agricultural crop productivity but also for the safety of the environment and public health. They are produced by plants, insects, and several microorganisms, which utilize them for survival and maintenance of defense mechanisms, as well as for growth and development. They are easily biodegradable, often times species-specific and also sometimes less toxic (or nontoxic) on other non-target organisms or species, an important consideration for alternate approaches of pest control. Several of the compounds, especially those produced by crop plants and other organisms, are consumed by humans and livestock, and yet appear to have no detrimental effects. They appear to be safe and will not contaminate the environment. Hence, they will be readily accepted for use in pest control by the public and the regulatory agencies. These natural compounds occur in nature only in trace amounts and require very low dosage for pesticide use. It is hoped that the knowledge gained by studying these compounds is helpful for the development of new pest control methods such as their use for interference with hormonal life cycles and trapping insects with pheromones, and also for the development of safe and biodegradable chemicals (e.g., pyrethroid insecticides). Undoubtedly, the costs are very high as compared to the presently used pesticides. But hopefully, these costs would be compensated for by the benefits derived through these natural pesticides from the lower volume of pesticide use and reduction of risks. Furthermore, the indirect or external costs resulting from pesticide poisoning, fatalities, livestock losses, and increased control expenses (due to the destruction of natural enemies and beneficial insects as well as the environmental contamination and pollution from chlorinated, organophosphorus, and carbamate pesticides) could be assessed against benefits vs. risks. The development and use of such naturally occurring chemicals could become an integral part of IPM strategies.

As long as they remain endogenously, several of the natural products presented in this handbook series serve as hormones, growth regulators, and sensory compounds for growth, development, and reproduction of insects, plants, and microorganisms. Others are useful for defense or attack against other species or organisms. Once these chemicals or their analogs and derivatives are applied by external means to the same (where produced) or different species, they come under the label "pesticides" because they contaminate the environment. Therefore, they are subject to regulatory requirements, in the same way the other pesticides are handled before they are used commercially. However, it is anticipated that the naturally occurring pesticides would easily meet the regulatory and environmental requirements for their safe and effective use in pest control programs.

A vast body of literature has been accumulated on natural pesticides during the last 2 or 3 decades; we have been assembling this information in these handbooks. We have limited our attempts to chemical and a few biological aspects concerned with biochemistry and physiology. Wherever possible, we tried to focus attention on the application of these compounds for pesticidal use. We hope that the first two volumes which dealt with theory and practice served as introductory volumes and will be useful to everyone interested in learning about the current technology that is being adapted from compound identification to the field trials. The subsequent volumes deal with the chemical, biochemical, and physiological aspects of naturally occurring compounds, grouped under such titles as insect growth regulators, plant growth regulators, etc.

In a handbook series of this type with diversified subjects dealing with plant, insect, and microbial compounds, it is very difficult to achieve either uniformity or complete coverage while putting the subject matter together. This goal was achieved to a large extent with the understanding and full cooperation of chapter contributors who deserve my sincere appreciation.

The editors of the individual handbooks relentlessly sought to meet the deadlines and, more importantly, to bring a balanced coverage of the subject matter, but, however, that seems to be an unattainable goal. Therefore, they bear full responsibility for any pitfalls and deficiencies. We invite comments and criticisms from readers and users as they will greatly help to update future editions. It is hoped that these handbooks will serve as a source book for chemists, biochemists, physiologists, and other biologists alike — those engaged in active research as well as those interested in different areas of natural products that affect the growth and development of plants, insects, and other organisms.

The editors wish to acknowledge their sincere thanks to the members of the Advisory Board for their helpful suggestions and comments. Their appreciation is extended to the publishing staff, especially Amy Skallerup, Melanie Mortellaro, and Sandy Pearlman for their ready cooperation and unlimited support from the initiation to the completion of this project.

N. Bhushan Mandava
Editor-in-Chief

FOREWORD

Pests of crops and livestock annually account for multi-billion dollar losses in agricultural productivity and costs of control. Insects alone are responsible for more than 50% of these losses.

For the past 40 years the principal weapons used against these troublesome insects have been chemical insecticides. The majority of such materials used during this period have been synthetic organic chemicals discovered, synthesized, developed, and marketed by commercial industry. In recent years, environmental concerns, regulatory restraints, and problems of pest resistance to insecticides have combined to reduce the number of materials available for use in agriculture. Replacement materials reaching the marketplace have been relatively few due to increased costs of development and the general lack of knowledge about new classes of chemicals having selective insecticidal activity.

In response to these trends, it is gratifying to note that scientists in both the public and private sectors have given significant attention to the discovery and evaluation of natural products as fertile sources of new insecticidal agents. Not only are these materials directly useful as insect control agents, but they also serve as models for new classes of chemicals with novel modes of action to attack selective target sites in pest species. Such new control agents may also be less susceptible to the cross resistance difficulties encountered with most classes of currently used synthetic pesticide chemicals to which insects have developed immunity.

Natural products originating in plants, animals, and microorganisms are providing a vast source of bioactive substances. The rapid development and application of powerful analytical instrumentation, such as mass spectrometry, nuclear magnetic resonance spectroscopy, gas chromatography, high performance liquid chromatography, immuno- and other bioassays, have greatly facilitated the identification of miniscule amounts of active biological chemicals isolated from natural sources. These new scientific approaches including biotechnology and tools are addressed and reviewed extensively in these volumes.

Some excellent examples of success in this research involve the discovery of insect growth regulators, especially the so-called juvenoids, which are responsible for control of insect metamorphosis, reproduction, and behavior. Pheromones which play essential roles in insect communication, feeding, and sexual behavior represent another important class of natural products holding great promise for new pest insect control technology. Another exciting approach is the development, commercialization, and use of safe, effective, naturally occurring microorganisms that have been formulated into microbial insecticides for the suppression and control of insect pests. All of these are discussed in detail in Volumes dealing with insects.

It is hoped that the scientific information provided in these volumes will serve researchers in industry, government, and academia, and stimulate them to continue to seek even more useful natural materials that produce effective, safe, and environmentally acceptable materials for use against insect pests affecting agriculture and mankind.

Orville G. Bentley
Assistant Secretary
Science and Education
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PREFACE

Our current generation realized a dream initially envisioned and prestructured by our predecessors. There has always been an expectation that disease-producing microorganisms of insects (i.e., entomopathogens) could be used to control insect pests. This expectation can be found in the descriptions of insect maladies from early Greek and Roman literature to the first conceptualization by Agostino Bassi (1834) that a microorganism (a fungus, *Beauveria bassiana*) can cause a disease (in silkworms).

Although Pasteur and LeConte both suggested (ca. 1874) that microorganisms might be used to control insect pests, the first concerted attempt to actually use an entomopathogen (a fungus, *Metarhizium anisopliae*) to control an insect pest (wheat cockchafer and sugar beet curculio) was demonstrated by E. Metchnikoff and I. Krassilstchik (ca. 1879). No major advancement occurred thereafter until the 1940s when R. T. White and S. R. Dutky demonstrated that a milky-disease bacterium (*Bacillus popilliae*) could be mass produced and effectively used to control grubs of the Japanese beetle. Significant major advancements, however, have occurred within the last three decades in the development, registration, commercialization, and use of a microbial insecticide (i.e., insect control agents formulated from microorganisms or their products). At least one of each major type of microorganism was developed and registered as a commercial microbial insecticide during this period. These registrations include the commercialization of a bacterium, *B. thuringiensis* (1961) and the first-time labeling and commercialization of a virus, *Baculovirus heliothis* (1974); a protozoan, *Nosema locustae* (1980); and a fungus, *Hirsutella thompsonii* (1981). If number of trade name products is considered then there are about three dozen commercial microbial pesticides available today. As one specific example...varieties of *B. thuringiensis* are used to formulate at least a dozen different commercial products for the control of either mosquitoes, beetles, caterpillars, spider mites, or lygus bugs.

The characterization and documentation of these significant advancements in the development and use of entomopathogens are the objectives of this two-part treatise on Microbial Insecticides. Scientists and others interested in an extensive review and bibliography will find these handbooks ideal both as a source of information and as a reference source. Authoritative researchers in their respective specialities have followed a common outline and format to bring consistent, thorough coverage to each presentation. Part A deals with entomogenous protozoa and fungi; Part B treats the entomogenous bacteria and viruses. Each presentation begins with an historical synopsis of the group and concludes with a glance into the future. Between these two extremes are discussions of potential candidates for development into microbial insecticides; specificity and virulence; stability and persistence in the environment; methods of production; and specific examples of uses of microorganisms as microbial insecticides. The text includes synoptic tables and is illustrated with photographs and drawings that provide excellent pictorial examples of each group of microorganism.

Carlo M. Ignoffo
Columbia, Missouri

ACKNOWLEDGMENT

The editor expresses his heartfelt appreciation to all the contributing authors for their patience and diligence. The editor also expresses his sincere gratitude to Flori Ignoffo, Dolores Reddick, and the many who assisted in any way in either the preparation, editing, reviewing, or proofing of the texts. Other specific acknowledgments by name are included by the authors within each section. Lastly we express out thanks to the staff of CRC Press, Inc. for all their untiring efforts.

DEDICATION

These volumes are dedicated to all scientists and their support groups, be they technical, familial, or spiritual, that have contributed to the development of microbial insecticides.

THE EDITOR-IN-CHIEF

N. Bhushan Mandava, holds B.S., M.S., and Ph.D. degrees in chemistry and has published over 150 papers including two patents, several monographs and reviews, and books in the areas of pesticides and plant growth regulators and other natural products as well as analytical instrumentation. As editorial advisor, he has edited three special issues on countercurrent chromatography for the *Journal of Liquid Chromatography*. He is now a consultant in pesticides and drugs. Formerly, he was associated with the U.S. Department of Agriculture and the Environmental Protection Agency as Senior Chemist. He has been active in several professional organizations, was President of the Chemical Society of Washington, and serves as Councilor of the American Chemical Society.

THE EDITOR

Carlo M. Ignoffo, Ph.D. is an Insect Pathologist and Director of the Biological Control of Insects Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Columbia, Missouri, and also holds an appointment as Adjunct Professor, Department of Entomology, University of Missouri-Columbia.

Dr. Ignoffo received his B.S. degree in Zoology from Northern Illinois University in 1950 and his M.S. and Ph.D. in Entomology in 1954 and 1957, respectively, from the University of Minnesota. He served in the U.S. Army, during the period 1954—1956 (Chemical Corps) conducting research on vectors of agents infectious to man and animals. Since the completion of his doctorate in 1957, he has been involved in teaching, research, and research leadership as Associate Professor of Entomology-Biology from 1957—1959 (Iowa Wesleyan College); a Research Entomologists/Pathologist from 1959—1965 (Entomology Research Division, USDA); a Research Pathologist and Administrator in industry from 1965—1971 (Bioferm/International Minerals and Chemical Corporation); and as a Research Leader/Laboratory Director (in his current appointment) since 1971.

Dr. Ignoffo is a charter member of the Society for Invertebrate Pathology and is or was a member of the American Association for the Advancement of Science, Entomological Society of America, American Institute of Biological Sciences, International Organization for Biological Control, and the Tissue Culture Association of America. He served as a technical advisor and research consultant to industry as well as International and National policy-making groups concerned with the development, production, use, and safety of microbial insecticides.

Research accomplishments are documented by authorship or coauthorship of more than 230 articles in scientific journals, including book chapters and review articles. He is a patentee in the field and was responsible for the isolation, commercialization, and registration of the world's first viral commercial pesticide. Dr. Ignoffo's life-time research focus has been in the development and use of microbial insecticides, especially viral insecticides. His current major research interest is in the characterization and manipulation of the genome of insect viruses in order to increase their ease of production, effectiveness, and persistence as viral insecticides.

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ENTOMOGENOUS PROTOZOA

Wayne M. Brooks

INTRODUCTION

The protozoa are a diverse and heterogeneous group of microorganisms. Many are associated with insects in relationships ranging from commensalistic to pathogenic. Those protozoa pathogenic for insects will be discussed with emphasis on species with demonstrated or promising potential as microbial insecticides. Unlike many of the entomopathogenic bacteria, viruses, or fungi, few of the entomogenous protozoa are highly virulent or fast acting. Most species produce chronic infections characterized by a general debilitation of the host. Thus, protozoa are most considered as candidates for long-term application or introduction programs and offer little potential as short-term, quick-acting microbial insecticides.[1-5]

The potential for utilizing protozoa as microbial insecticides must be visualized in the context of their general characteristics as pathogens of insects. Infection is usually initiated with peroral ingestion of spores, cysts, or other stages by a larval stage of the host. Some species also are commonly transmitted on the surface of the egg (transovum) or within the egg (transovarian) of their host. In special cases, some protozoa are transmitted mechanically via the ovipositional activities of hymenopterous parasites. Infections are generally sublethal and chronic in nature and pathognomonic signs or symptoms are seldom exhibited by infected hosts. Most infections are characterized by such nonspecific signs and symptoms of disease as sluggishness, irregular growth, loss of appetite, malformed larvae, pupae, or adults, or adults with reduced vigor, fecundity, and longevity. The period of infection may only be slightly shorter than the normal life span of the host, and in some cases larval or pupal periods are lengthened. While infected hosts may die prematurely, the actual cause of death is difficult to determine. Competition for nutrients may be important where the extracellular phase of protozoan development is extensive. However, this possibility as well as the possibility that some protozoa produce toxins has not been thoroughly investigated. Death of the host is most likely the result of mechanical destruction of cells by developing stages of the protozoan.

With few exceptions, most protozoa must be produced in vivo using whole-organism technology. This is inherently more difficult and expensive than is the production of bacteria and fungi using fermentation technology. Long-term storage of infective stages of protozoa is difficult and persistence of unprotected stages, under field conditions, is of short duration. While the host range of only a few species has been examined extensively, it has become increasingly evident that most species are not as host specific as was once generally considered. The host spectrum of a few species, however, is sufficiently broad to encourage their production as microbial insecticides. Limited assessment of infectivity to nontarget organisms and the obvious need for increased research in the areas of formulation and application technology mark the infant state of use of protozoa as microbial insecticides. Additional comprehensive and general discussions of protozoa as pathogens of insects are presented in earlier reviews by Paillot,[6] Steinhaus,[7,8] Aoki,[9] Lipa,[10,11] Weiser,[12,13] and Brooks.[14]

HISTORICAL BACKGROUND

The entomogenous protozoa played a prominent role in the development of the field of insect pathology although most of the limited efforts to utilize protozoa as microbial control agents have been conducted since the early 1950s. Reports in the early 1800s of observations and descriptions of gregarines associated with insects are discussed briefly by Steinhaus[15] and similar early reports of entomogenous flagellates are presented by Wallace.[16] More significantly, Pasteur[18] (along with the earlier observations of Bassi[17] on the muscardine disease of silkworms caused by a fungus) helped establish insect pathology as a science through his classical study of a protozoan infection (pebrine) of the silkworm *Bombyx mori*. Pasteur showed that the peculiar microscopic corpuscles (spores) seen by earlier students of silkworm diseases were the cause of the disease and made observations that led to a method for obtaining disease-free silkworms. While the true nature of the parasite as a protozoan was unknown to Pasteur or to Naegeli[19] who named the parasite *Nosema bombycis*, subsequent observations by Balbiani[20] and Stempell[21] established the identity of the parasite as a protozoan in the order Microsporida. It is also significant that Pasteur[22] made one of the first definite suggestions that microorganisms might be used to control insects. He suggested the use of "les corpuscles" of pebrine against the grape phylloxera, a pest threatening grape production in France at the time. More detailed accounts of Pasteur's contributions to insect pathology are presented by Steinhaus.[8,15]

Many protozoa were described from insects and other invertebrates in the early 1900s and some, such as *Ameson pulvis* from the green crab *Carcinus maenas*, were suggested as possible biological control agents.[23] However, the first apparent attempt to use a protozoan as a microbial control agent was carried out by Taylor and King.[24] Utilizing cyst-containing feces collected from grasshoppers infected with the amoeba *Malameba locustae*, they applied the amoeba in a mixture with bran and molasses along roads and fences. A low incidence of infection was found in grasshoppers after 8 weeks but insufficient data were collected to determine if any control was achieved. Serious attempts were not made until the early 1950s to use protozoa as microbial control agents, perhaps influenced in part by the chronic nature of most protozoan infections and the often erratic results in tests involving some entomogenous fungi and bacteria. Limited attempts utilizing various microsporidia were carried out in the U.S. by Hall[25] and Zimmack et al.[26] and in Czechoslovakia by Weiser and Veber.[27,28] The first extensive tests to utilize protozoa as microbial control agents were carried out by McLaughlin and his associates in the mid 1960s[29-33] and involved the neogregarine *Mattesia grandis* and the microsporidium *Nosema gasti* against the cotton boll weevil, *Anthonomus grandis*, in the southern U.S. More recently, the mass production and successful use of the microsporidium *Nosema locustae* against grasshoppers on rangelands[34-38] led to registration of this protozoan in 1980 by the U.S. Environmental Protection Agency. This was the first protozoan commercially produced as a microbial insecticide in the U.S. Earlier reviews on protozoa as microbial control agents have been presented by McLaughlin,[1] Pramer and Al-Rabiai,[3] Tanada,[39] Brooks,[4] Henry,[40] Wilson,[41] and Canning.[5]

TAXONOMY

As a heterogeneous group of essentially single-celled, eukaryotic organisms, the protozoa have been usually placed together as a matter of convenience rather than as a natural grouping of related organisms. They have been treated classically as a single phylum and traditionally divided into two subphyla with five classes based on locomotory organelles. However, the classification of the protozoa has undergone considerable revision at the suprafamilial level in recent years.[42,43] In the most recent report of the Committee on Systematics and Evolution of the Society of Protozoologists,[43] the protozoa are treated as a subkingdom and seven phyla are recognized, five of which contain entomogenous protozoa. A synopsis of this classification scheme showing the major taxa that include entomogenous protozoa is presented in Table 1. In the subsequent discussion, emphasis will be placed on those taxa which include species pathogenic for insects. Useful keys to most of the common genera of entomogenous protozoa are provided by Poinar and Thomas.[44,44a]

Table 1
CLASSIFICATION SCHEME OF ENTOMOGENOUS PROTOZOA

Taxa[a]	Representative genera
Phylum Sarcomastigophora	
Subphylum Mastigophora	
Class Zoomastigophorea	
Order Kinetoplastida	*Herpetomonas, Crithidia, Leptomonas*
Order Retortamonadida	*Retortamonas*
Order Diplomonadida	*Octomitus*
Order Oxymonadida	*Oxymonas, Pyrosonympha*
Order Trichomonadida	*Trichomonas, Devescovina*
Order Hypermastigida	*Trichonympha, Joenia*
Subphylum Sarcodina	
Superclass Rhizopoda	
Class Lobosea	
Subclass Gymnamoebia	
Order Amoebida	*Malpighiella, Malameba, Malpighamoeba*
Phylum Apicomplexa	
Class Sporozoea	
Subclass Gregarinia	
Order Eugregarinida	*Gregarina, Ascogregarina*
Order Neogregarinida	*Mattesia, Farinocystis, Ophryocystis*
Subclass Coccidia	
Order Eucoccidiida	*Adelina, Legerella, Barrouxia*
Phylum Microspora	
Class Microsporea	
Order Minisporida	*Chytridiopsis, Hessea*
Order Microsporida	*Nosema, Pleistophora, Amblyospora*
Phylum Ascetospora	
Class Stellatosporea	
Order Balanosporida	*Haplosporidium*
Phylum Ciliophora	
Class Kinetofragminophorea	
Subclass Vestibulifera	
Order Trichostomatida	*Balantidium*
Subclass Suctoria	
Order Suctorida	*Discophrya, Rhynchophrya*
Class Oligohymenophorea	
Subclass Hymenostomatia	
Order Hymenostomatida	*Tetrahymena, Lambornella*
Subclass Peritrichia	
Order Peritrichida	*Epistylis, Opercularia*
Class Polyhymenophorea	
Subclass Spirotrichia	
Order Heterotrichida	*Nyctotherus*

[a] Compiled primarily from Reference 43.

The phylum Sarcomastigophora includes those protozoa referred to as flagellates and amoebae which generally possess flagella and/or pseudopodia (sometimes both types of organelles are present) and only a single type of nucleus. They typically do not form spores. Flagellates (subphylum Mastigophora) typically possess one or more flagella and usually reproduce asexually by longitudinal binary fission. The entomogenous flagellates are included in the class Zoomastigophorea characterized by the lack of chromatophores and being predominantly parasitic. The largely mutualistic and commensalistic flagellates which occur in the intestinal track of termites, roaches, and a few other insects are included in the orders

Retortamonadida, Diplomonadida, Oxymonadida, Trichomonadida, and Hypermastigida. Most of the entomogenous, pathogenic flagellates are included in the order Kinetoplastida, family Trypanosomatidae, and are generally referred to as trypanosomatids. These flagellates are usually elongate and slender in shape and possess a single flagellum either free or attached to the body by an undulating membrane. The flagellum arises within a reservoir and is associated with a contractile vacuole and a Feulgen-positive kinetoplast. While a large number of trypanosomatids are associated with insects, relatively few are pathogenic. The strictly entomogenous trypanosomatids are included in the genera *Herpetomonas, Crithidia, Blastocrithidia,* and *Rhynchoidomonas.* Many of the flagellates of the genus *Leptomonas* are also entomogenous, and other species are also known from other invertebrates including protozoa, nematodes, and mollusks. The digenetic flagellates of the genera *Leishmania* and *Trypanosoma* have both an invertebrate and vertebrate host while those in the genus *Phytomonas* have a plant and invertebrate host. A list of representative species of the entomogenous trypanosomatids is included in Table 2. An extensive list of the entomogenous trypanosomatids from insects and a review of the systematics of the group was presented by Wallace.[16] A more recent review on the biology of these flagellates from arthropods has also been published.[45]

Amoebae (subphylum Sarcodina) possess pseudopodia, a naked body, and reproduce asexually by binary or multiple fission, often in cysts. Flagella, when present, are usually restricted to developmental stages and none are present in the entomogenous amoebae. Nearly all of the entomogenous amoebae occur in the families Amoebidae and Endamoebidae, order Amoebida characterized by a single nucleus and lobose pseudopodia. The cytoplasm is generally divided into a granular endoplasm filled with inclusions and a hyaline ectoplasm. The strictly entomogenous amoebae belong to the family Amoebidae and are represented by the genera *Malameba, Malpighamoeba,* and *Malpighiella.* Among the parasitic Endamoebidae, a few entomogenous species are known in the genera *Entamoeba, Endamoeba, Endolimax,* and *Dobellina.* These amoebae are considered as commensals of the alimentary tract of various insects, primarily cockroaches. However, Purrini and Halperin[76a] recently described an *Endamoeba* sp. in the gut of the bark beetle, *Orthotomicus erosus,* that they suggested was harmful despite the lack of external symptoms in heavily infected adults. Representative species of entomogenous amoebae are shown in Table 2, only two of which, *Malameba locustae* and *Malpighamoeba mellificae,* produce significant pathological effects in insects.

As the causative agent of amoebic disease of the honey bee, *Apis mellifera, M. mellificae* is not a candidate microbial control agent, since the honey bee is a beneficial insect. However, *Malameba locustae* has been shown to be infectious for a wide range of grasshopper species with sufficient virulence to be considered as a potential microbial control agent. The taxonomic status of this species and the other entomogenous amoebae is in need of re-evaluation and probable revision. Most authorities, however, have continued to accept the placement of the strictly entomogenous genera *Malameba, Malpighamoeba,* and *Malpighiella* within the family Amoebidae, although Harry and Finlayson[79] suggested that *Malameba locustae* may belong to the family Schizopyrenidae based on preliminary observations on ultrastructure. Purrini[80] recently described another entomogenous amoeba, *Malameba scolyti,* from the bark beetles *Dryocoetes autographus* and *Hylurgops palliatus.*

The phylum Apicomplexa contains those protozoa typically referred to as sporozoa and are characterized by an apical complex (visible with the electron microscope) present at some stage which generally consists of polar ring(s), rhoptries, micronemes, and conoid and subpellicular microtubules. Microspores are generally also present at some stage, cilia are absent, sexuality is by syngamy, and all species are parasitic. The entomogenous sporozoa belong to the class Sporozoa characterized by a well developed apical complex, sexual and asexual reproduction, and spores or oocysts containing infective sporozoites produced by

Table 2
A LIST OF THE PRINCIPAL GROUPS AND GENERA OF ENTOMOGENOUS PROTOZOA, ALONG WITH REPRESENTATIVE SPECIES AND SELECTED REFERENCES[a]

Group	Genus	Representative species	Selected Ref.[b]
Trypanosomatids	*Blastocrithidia*	*caliroae*	46
		gerridis	47, 48
		leptocoridis	49, 50
		raabei	51
	Crithidia	*acanthocephali*	52
		fasciculata	16, 53, 54
		luciliae	55, 56
		oncopelti	57, 58
		tabani	59
	Herpetomonas	*ludwigi*	60, 61
		muscarum	16, 62, 63
		swainei	64
	Leptomonas	*ctenocephali*	65, 66
		oncopelti	57, 67
		pyrrhocoris	68—70
		serpens	71
		seymouri	72
	Rhynchoidomonas	*drosophilae*	73, 74
		luciliae	75
		siphunculinae	76
Amoebae	*Amoeba*	*chironomi*	81
	Dobellina	*mesnili*	82, 83
	Malameba	*locustae*	24, 77, 79, 84, 85
		scolyti	80
	Malpighamoeba	*mellificae*	86, 87
	Malpighiella	*refringens*	88
Eugregarines	*Ascogregarina*	*armigerei*	109
		barretti	110
		brachyceri	110a
		chagasi	111
		clarki	112
		culicis	109, 110, 113
		galliardi	114
		lanyuensis	109
		legeri	115
		mackiei	116
		taiwanensis	109
		tripteroidesi	117
Neogregarines	*Caulleryella*	*apiochaetae*	118
		pipientis	119
	Coelogregarina	*ephestiae*	106, 107
		orchopiae	106, 120
	Farinocystis	*tribolii*	121
	Gigaductus	*agoni*	122
		anchi	103, 123
		steropi	124
	Lipocystis	*polyspora*	125
	Lipotropha	*macrospora*	126
		microspora	126
	Lymphotropha	*tribolii*	102
	Machadoella	*triatomae*	127
	Mattesia	*dispora*	128

Table 2 (continued)
A LIST OF THE PRINCIPAL GROUPS AND GENERA OF ENTOMOGENOUS PROTOZOA, ALONG WITH REPRESENTATIVE SPECIES AND SELECTED REFERENCES[a]

Group	Genus	Representative species	Selected Ref.[b]
		grandis	129—131
		trogodermae	106, 132
	Menzbieria	*chalcographi*	133
	Orphryocystis	*dendroctoni*	134
		elektroscirrha	135
	Schizocystis	*gregarinoides*	136
		legeri	137
	Syncystis	*mirabilis*	138
	Tipulocystis	*maximae*	60
Coccidia	*Adelina*	*cryptocerci*	139
		sericesthis	140
	Barrouxia	*bellostomatis*	141
		schneideri	142
	Chagasella	*alydi*	143
	Ithania	*wenrichi*	144
	Legerella	*hydropori*	145
		parva	146
	Rasajeyna	*nannyla*	147
Microsporidia[c]	*Amblyospora*	*californica*	154, 155
		inimica	155, 156
		minuta	155, 157
		opacita	155, 158
	Auraspora	*canningae*	159
	Bohuslavia	*asterias*	159a, 159b
		simulii	159b, 159c
	Burenella	*dimorpha*	160, 161
	Buxtehudea	*scaniae*	162
	Campanulospora	*deliae*	162a
	Caudospora	*pennsylvanica*	163, 164
		polymorpha	165, 166
		simulii	167, 168
	Chapmanium	*cirritus*	155
		dispersus	155a
	Chytriodiopsis	*socius*	169, 170.
		typographi	134, 171
	Cougourdella	*polycentropi*	172
		rhyacophilae	173
	Culicospora	*magna*	149, 157, 174
	Culicosporella	*lunata*	149, 175
	Cylindrospora	*chironomi*	149c
		fasciculata	149d
	Cystosporogenes	*operophterae*	175a
	Duboscqia	*chironomi*	176
		coptotermi	177
		legeri	178, 179
	Episeptum	*inversum*	179a
	Evlachovaia	*chironomi*	149c
	Golbergia	*spinosa*	149, 180
	Gurleya	*aeschnae*	181
		chironomi	182
		legeri	183
	Hazardia	*milleri*	149, 184

Table 2 (continued)
A LIST OF THE PRINCIPAL GROUPS AND GENERA OF ENTOMOGENOUS PROTOZOA, ALONG WITH REPRESENTATIVE SPECIES AND SELECTED REFERENCES[a]

Group	Genus	Representative species	Selected Ref.[b]
	Helmichia	*aggregata*	185
	Hessea	*squamosa*	186a
	Hirsutusporos	*austrosimulii*	186d
	Hyalinocysta	*chapmani*	155
		expilatoria	186b
	Issia	*globulifera*	186c
		trichopterae	149, 167
	Janacekia	*debaisieuxi*	167a, 167b
	Jirovecia	*brevicauda*	149, 187
	Mitoplistophora	*angularis*	188
	Microsporidium[d]	*goeldichironomi*	189
		hyphantriae	28, 152, 190
	Neoperezia	*chironomi*	191
	Nosema	*algerae*	192—194
		apis	195—198
		bombycis	19, 199, 200
		fumiferanae	201, 202
		gasti	203, 204
		locustae	205—207
		pyrausta	208—213
	Octosporea	*carloschagasi*	214
		muscaedomesticae	215, 216a
	Orthosoma	*operophterae*	216b, 216c
	Parathelohania	*anophelis*	155, 157, 217
		legeri	155, 217—219
		obesa	155, 157, 217, 220
	Pegmatheca	*simulii*	155
	Pilosporella	*chapmani*	155
		fishi	155
	Pleistophora	*californica*	221
		kudoi	222, 223
		schubergi	224—227
	Polydispyrenia	*simulii*	227a, 250
	Resiomeria	*odonatae*	149d
	Semenovaia	*chironomi*	149c
	Stempellia	*mutabilis*	228, 229
	Striatospora	*chironomi*	149c
	Systenostrema	*tabani*	155
	Telomyxa	*glugeiformis*	228, 230
		orae	230a
	Thelohania[e]	*fibrata*	231, 232
		pristiphorae	233
		pyriformis	157, 220
	Toxoglugea	*chironomi*	234, 235
		variabilis	236
		vibro	237, 238
	Trichoduboscqia	*epeori*	239—241
	Tuzetia	*ecdyonuri*	167a, 242
		schneider	167a, 228
	Unikaryon	*bouixi*	243a
		minutum	243

Table 2 (continued)
A LIST OF THE PRINCIPAL GROUPS AND GENERA OF ENTOMOGENOUS PROTOZOA, ALONG WITH REPRESENTATIVE SPECIES AND SELECTED REFERENCES[a]

Group	Genus	Representative species	Selected Ref.[b]
	Vairimorpha	*necatrix*	244, 245
		plodiae	246—248
	Vavraia	*culicis*	149, 167, 249, 250
	Weiseria	*laurenti*	251, 252
		sommermanae	164
Ciliates	*Lambornella*	*clarki*	258, 259
		stegomyiae	261
	Tetrahymena	*chironomi*	257, 262
		rotunda	260a
		dimorpha	260b
		sialidos	260i

[a] Exclusive of the commensalistic flagellates primarily associated with termites and blattids, and the predominantly commensalistic eugregarines of coleopterans, orthopterans, dipterans, and other insects.
[b] The selected references generally refer to papers dealing with the description and/or life cycle of the species or in some cases to a major taxonomic treatise which indicates the current status of the species.
[c] Except for the new general and species described recently, an annotated list of the species of microsporidia is included in the comprehensive treatise of Sprague.[152] Many species are also reviewed in the monograph of Weiser.[212]
[d] A collective group without established attributes in which identifiable species can be placed provisionally because their generic status is uncertain.[148]
[e] According to Hazard and Oldacre,[155] this genus is represented by species found in decapod crustaceans. The entomogenous species included here are considered as being of doubtful status.

sporogony. A majority of the described species of entomogenous sporozoa are included in the subclass Gregarinia and are referred to as gregarines. They are characterized by mature gamonts which are extracellular and relatively large, attachment organelles (the mucron or epimerite) may be present, gametes generally similar (isogamous), gamonts which undergo syzygy, the formation of oocysts by zygotes within gametocysts, and a life cycle characteristically consisting of gametogony and sporogony. Of the approximately 1400 known species,[89] about 93% belong to the order Eugregarinida, most of which are harmless commensals of the digestive tracts of insects. The gregarines in this order are characterized by a life cycle involving gametogony and sporogony but not merogony (i.e., there is no asexual reproduction). Mobile species move by gliding or undulation of longitudinal ridges of the body surface. As this group of entomogenous protozoa is exceptionally large in number (1300 species in 195 genera and 29 families) and is largely commensalistic or of low virulency to insects, no attempt will be made to delineate the families, genera, or species involved. Along with the early taxonomic treatments on eugregarines by Watson[90] and Kamm,[91] checklists and revisions of many genera and species of this order have been provided in a recent series of papers by Levine.[92-97] Grassé[98] provides a classification scheme of the higher taxa, and a recent general discussion of gregarines is provided by Manwell.[99]

Among the eugregarines considered to be somewhat pathogenic to their hosts, the species of the genus *Ascogregarina* have received the most attention. These eugregarines (see Table 2) are parasites of mosquitoes and other insects,[94] and at least one species, *A. culicis,* has been evaluated as a potential microbial control agent for mosquitoes.