INSECT DISEASES

VOLUME I

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Edited by George E. Cantwell

INSECT PATHOLOGY LABORATORY
PLANT PROTECTION INSTITUTE
AGRICULTURAL RESEARCH SERVICE, USDA
BELTSVILLE, MARYLAND

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DR. SAMSON R. DUTKY

This book is dedicated to Sam Dutky, a foremost pioneer in the field of Insect Pathology and Microbial Control. In 1937 as a graduate student at Rutgers University, he discovered and named the bacilli which are responsible for the milky diseases of the Japanese beetle; more importantly he recognized their potential as microbial control agents. Under Dr. Dutky's direction and leadership as a Research Microbiologist with the U.S. Department of Agriculture, the milky disease program that led to the control of the Japanese beetle in the northeastern part of the United States became a reality, and it stands today as the most outstanding example of microbial control of insects yet devised by man.

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CONTRIBUTORS TO VOLUME I

JAMES V. BELL,* Entomology Research Division, Agricultural Research Service, USDA, Charleston, South Carolina

WAYNE M. BROOKS, Department of Entomology, North Carolina State University, Raleigh, North Carolina

ROBERT M. FAUST, Insect Pathology Laboratory, Plant Protection Institute, Agricultural Research Service, USDA, Beltsville, Maryland

GERALD M. THOMAS, Department of Entomological Sciences, Division of Entomology and Parasitology, University of California, Berkeley, California

JAMES L. VAUGHN, Insect Pathology Laboratory, Plant Protection Institute, Agricultural Research Service, USDA, Beltsville, Maryland

* Present address: U.S. Delta States Agricultural Research Center, Bioenvironmental Insect Control Laboratory, Stoneville, Mississippi.

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PREFACE

Aside from the purely academic desire to obtain knowledge, there are two fundamental reasons for studying insect diseases: (1) to learn how to manipulate these diseases so as to reduce pest insect populations and (2) to control natural diseases in beneficial insects, experimental insect colonies, and mass rearings.

The field of insect pathology has grown greatly during the past 20 years; today over 900 scientists are listed in the "Directory for Invertebrate Pathology," and the subject is taught in nearly 40 colleges and universities in English-speaking countries. It now draws on many disciplines and specialists to maintain its current impetus. Since no one individual is quite as competent in this broad spectrum of studies as is a specialist with expertise in one area, eleven practicing scientists have contributed to the formation of this text.

The text is designed to assist the upper-division or graduate student in his first exposure to the field of insect pathology. The minimum prerequisites are a course or two in entomology and one in microbiology. The subject areas covered in the text include diagnoses, and microbial diseases caused by viruses, rickettsiae, bacteria, fungi, protozoans, and nematodes. Symbiotic relationships are discussed as well as amicrobial pathologies associated with genetic and endocrine imbalances or exposure to radiation or chemicals.

Since classical pathology requires laboratory studies, 23 laboratory exercises are included and may be found at the end of most chapters. Although some schools may find a few of these exercises beyond their capabilities because of the lack of needed equipment, most exercises do not require sophisticated or expensive set-ups.

Because of the descriptive nature of pathology all contributing authors were urged the very liberal use of photographs and other illustrative material; over 100 figures are included in the text. Although no attempt was made to present a thorough and complete bibliography, a total of over 900 selected references are included. A glossary of terms most frequently used in the field of insect pathology is also provided.

I wish to thank the many individuals who kindly provided photographic prints and who permitted their reproduction in this textbook.

George E. Cantwell

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Chapter 1

DIAGNOSTIC TECHNIQUES

Gerard M. Thomas
Department of Entomological Sciences
Division of Entomology and Parasitology
University of California
Berkeley, California

I. INTRODUCTION

In a logbook entitled "Accession Numbers for Specimens Submitted to Insect Pathology Lab for Examination," the following entry, written by the late Dr. Edward A. Steinhaus (1914-1969), is found:

"Accession Number 1, July 3, 1945. Mealybugs sent up from Riverside by Glenn Finney. About ten specimens each of the following species: (1) Pseudococcus citri, (2) Pseudococcus longispinus, (3) Pseudococcus bakeri, (4) Pseudococcus gahani. Triturated specimens to be examined as to microbial flora."

This, essentially, was the beginning of the Diagnostic Service for the Diseases of Insects at the Laboratory of Insect Pathology, Department of Entomology, University of California, Berkeley. Although the Diagnostic Service was not formally instituted until January, 1951 [1] a total of 575 accessions had been received and processed by that time. This same logbook now records over three thousand accessions which have come from entomologists, agriculturists, students, and biologists in general, from nearly all parts of the United States, and from many foreign countries representing every continent except Antarctica.

Man has been aware that insects suffer from disease since before the time of Aristotle, but much of this awareness was restricted to domesticated insects, such as the honey bee and the silkworm. Indeed, Aristotle records several diseases of the honey bee. However, it was not until 1834 when Augustino Bassi, from the town of Lodi in Italy, showed that the white muscardine disease of the silkworm was caused by a fungus. This was the first time a microorganism was shown to be the cause of a disease in insects. In 1870, Louis Pasteur published the results of his studies on pebrine in the silkworm, proving the cause of this disease to be a protozoan. It was during this work in Pasteur's laboratory that a microscope was first used in the diagnosis of infectious diseases [2]. Since then much progress has been made in the techniques for studying microbial etiologies of disease.

In insect pathology, many techniques and procedures have been borrowed from related fields such as virology, bacteriology, mycology, protozoology, nematology, histology, electron microscopy, etc., and modified as needed to suit the work in this particular scientific discipline. On the other hand, certain techniques have been developed for procedures specific to insect pathology. In diagnostic work, however, the main techniques used are essentially those basic to entomology and microbiology, and the main tool is the microscope. These serve to enhance and extend the powers of observation of the diagnostician. Steinhaus [3] refers to diagnostics as a combination of art and science, alluding to the quotation that art is "knowledge made efficient by skill." Certainly a variety of scientific knowledge is necessary in diagnosing the diseases of insects, and the efficiency of the diagnosis depends on the skill with which this knowledge is applied. Through experience, an individual gains skill in the application of knowledge. The student may experience difficulty at first in attempting to diagnose diseases of insects, but with practice and

increased knowledge of insect pathology, these difficulties will gradually be overcome.

Webster's Third New International Dictionary gives the following definition of disease: "an impairment of the normal state of the living animal or plant body or any of its components that interrupt or modify the performance of the vital functions, being a response to environmental factors (as malnutrition, industrial hazards, or climate), to specific infective agents (as worms, bacteria, or viruses), to inherent defects of the organism (as various genetic anomalies), or to combinations of these factors." Or, more simply stated, disease is any departure from the normal healthy state of a living organism, whatever its cause.

Steinhaus and Martignoni [4] offer this definition of diagnosis: "to distinguish one disease from another. The determination of a disease from its signs, symptoms, etiology, pathogenesis, physiopathology, morphopathology, etc. Also, the decision reached." Succinctly then, diagnosis may be thought of as the process of determining a disease and its cause (etiology).

Diseases can be classified as nonmicrobial or noninfectious diseases (conditions in which a living microorganism is not involved), and microbial or infectious diseases (conditions resulting from the presence of a living microorganism). The nonmicrobial diseases include injuries due to mechanical trauma, adverse physical environmental factors (e.g., drought, freezing, overheating, etc.), parasitization and predation; toxic effects of chemical agents (e.g., insecticides); nutritional deficiencies, deranged physiology and metabolism; nongenetic congenital abnormalities; and inherited or genetic abnormalities. The foregoing nonmicrobial diseases, particularly genetic diseases, are more readily assessed and therefore diagnosed by an individual who is rearing, or in some way working closely with a particular species or group of insects and thus familiar with what constitutes normal specimens. Another individual, although competent in microbial diagnosis, may be unfamiliar with the particular insect in question, and may have difficulty in making a nonmicrobial diagnosis.

This chapter is concerned mainly with diagnosis of microbial diseases of insects, and is intended to introduce the student to the techniques of microbiology and microscopy used in making such diagnoses. Its aim is to provide the technical knowledge that will enable the student to develop skill in diagnosing a disease etiology to one of the major groups of insect pathogens e.g., viruses, rickettsia, bacteria, fungi, protozoa, or nematodes, or decide that the etiology may be nonmicrobial. More detailed information concerning each of these groups, as well as other types of pathologies, will be found in subsequent chapters of this text.

A. Major Groups of Insect Pathogenic Microorganisms

The importance of diagnosis to insect pathology is fundamental. This becomes rather obvious when considering that in order to study a disease, one must know its nature and cause. In order to study the role of a pathogen in the ecological life of an insect, or to assess its potential as a microbial control agent, the pathogen must be identified. It follows, therefore, that the identification of a pathogen is an essential element in the diagnosis of a microbial disease.

The first step in the identification of a pathogen is to determine to which one of the major groups of microorganisms it belongs. The following is a brief description of the major groups of insect pathogenic microorganisms.

1. Viruses

Viruses are submicroscopic, obligate, intracellular pathogens, which influence the host cell to replicate virus DNA or RNA (as the case may be) rather than the nucleic acids of the cell, and which require the living host cell for their reproduction. Generally speaking, viruses cannot be seen under the ordinary light microscope. However, some of the insect viruses are occluded in a proteinic inclusion that is visible under the microscope. Thus, the viruses causing diseases in insects may be separated into two main groups – the inclusion viruses and the noninclusion viruses. In the former group, at the end of their development, protein inclusion bodies are formed that contain the virus particles. The polyhedrosis viruses are characterized by inclusion bodies roughly polyhedron in shape, from 3 to 10 μm in diameter, and containing many virus particles. Polyhedrosis viruses developing in the nucleus of infected cells contain rod-shaped virus particles, whereas those developing in the cytoplasm contain spherical virus particles. The granulosis viruses are characterized by a single rod-shaped virus particle within a capsule-shaped inclusion body of less than 0.5 μm in length. Although the inclusion bodies can be detected under the light microscope, the virus particles themselves can be seen only with the aid of the electron microscope. The noninclusion viruses are spherical and develop in infected cells (most in the cytoplasm, but some in the nuclei) without the formation of a protein inclusion. In general, the route of infection for viruses is through the alimentary tract, although transovarian transmission may also occur. Viruses cannot be cultivated on artificial media.

2. Rickettsia

The insect pathogenic Rickettsia belong to the genus Rickettsiella. They are mainly intracellular pathogens about the size of granulosis inclusions

(0.3-0.5 μm in length), pleomorphic, sometimes forming chains of cells, and staining with Giemsa and Macchiavello's stains which differentiate them from inclusion viruses. They are not cultivable on ordinary bacteriological media. The route of infection is through ingestion.

3. Bacteria

Bacteria are unicellular microorganisms belonging to the class Schizomycetes; generally exhibit one of three forms — bacillus (rod), spirillum (spiral-shaped rod), or coccus (sphere); and are usually large enough to be seen under the ordinary light microscope. Many bacteria are associated with insects, particularly those common to the insect's environment, but relatively few are capable of infecting and killing a host that ingests them. However, many saprophytic species are capable of causing a fatal septicemia if they can gain entrance to the hemocoel. Since many bacteria are regularly associated with dead insects and take part in the decomposition of the cadaver, one must carefully evaluate bacteria isolated from insects. The normal route of infection is through the gut, but infection through wounds is also common.

4. Fungi

The most common and easily recognized characteristics of fungi infecting insects are the filamentous nature of the vegetative thallus, and the characteristic reproductive structures usually produced on the external surface of the dead host. There are notable exceptions, however, such as the internal cystlike sporangia or Coelomomyces in aquatic insects (e.g., mosquito larvae), and the yeastlike cells of Syngliocladium. Many saprophytic fungi, associated with insects, are capable of growing on a cadaver killed by some other etiological agent, and, as with the bacteria, one must carefully evaluate fungi isolated from or associated with dead insects. Normally, fungi invade the host through the integument, but there are a few (such as the Aspergilli) that usually infect through the gut wall.

5. Protozoa

The protozoa are a diversified phylum of single celled microorganisms, reproducing typically by binary fission, and placed in the animal kingdom. Members of all classes of protozoa are known to parasitize insects and their diversity of form and type of association is probably greater than in any other major group of insect pathogens. This group is so diversified that it would be difficult and probably unproductive to the student to attempt to generalize on it further, except to say that the Sporozoa (gregarines and coccidians), the Cnidospora (microsporidians),

and the Sarcomastigophora (amoeboflagellates) are the predominant groups of insect pathogens. The route of infection is typically through the alimentary tract.

6. Nematodes

Nematodes are metazoan organisms belonging to the phylum Nemathelminthes or roundworms, possessing a mouth and an intestinal canal. Many of the insect-pathogenic nematodes, such as the Mermithidae and the Gordiaceae, are visible with the naked eye, while others, such as the Neoplectanidae are easily detected under the dissecting microscope. The normal routes of infection are through the alimentary canal, or through the integument.

B. Safety of Insect Pathogens

When working with apparently diseased insects and attempting to make a diagnosis, one must be concerned with the safety of insect pathogens to oneself, and co-workers. For a long time many scientists have worked with a great variety of insect pathogens without becoming infected themselves. Consequently, these pathogens have generally become regarded as innocuous to higher forms of life. Although this assumption is probably true for most insect pathogens, it must be kept in mind that very few have been tested for pathogenicity or toxicity to man or other vertebrate animals. While recent studies [5] have shown the insect viruses to be probably the safest group, and the bacterium Bacillus thuringiensis Berliner and closely related varieties are also safe, there are other insect pathogens capable of causing infection in higher animals, including man. The bacteria Pseudomonas aeruginosa Migula and Serratia marcescens Bizio are such examples. Some Rickettsiae are known to infect mammals lethally. Of the fungi, Entomophthora coronata (Costantin) Kevorkian, has been identified as the cause of a phycomycosis in horses and man, but this is the only species of this genus known, so far, to cause infection in higher animals. Some fungi belonging to the genus Aspergillus can kill fowl, and cause a fatal lung disease (aspergillosis) in man. Although in the latter case, a very heavy inoculum is required to induce infection. Among the protozoa, microsporidial infections have been reported in man, but none of the protozoans involved have been identified as insect pathogens. In fact, protozoan pathogens of insects frequently occur in stored foods, but none of these have caused disease in man or domestic animals, indicating perhaps that these particular protozoa are not pathogens of vertebrate animals [5].

Whereas we may generally assume that insect pathogens are safe for man, the few exceptions noted are sufficient reason for advising caution when working with these microorganisms. It must also be kept in mind

that insects carry a great variety of phoretic microorganisms which may or may not be pathogenic for higher animals. Taking proper precautions in the laboratory to avoid personal contamination and to minimize general contamination should become a habit when dealing with unknown and potentially pathogenic microorganisms.

II. GENERAL LABORATORY PROCEDURES

A. Laboratory Cleanliness

Nowhere in laboratory work is it more important to take precautions against dust contamination than in a laboratory where pathological studies are conducted. Strict cleanliness of hands, work table, and apparatus is essential in pathological work. The laboratory table top should be wiped with a cloth moistened with a disinfectant, before and after the work is completed. In addition, the working area, and the entire room for that matter, should be kept as free as possible from dust collecting clutter. When conducting pathological studies, infectivity tests, studying the nature of isolated microorganisms, etc., purity of culture is essential. No matter how careful one is, some contamination is bound to be encountered, but this can be minimized by following strict procedures of laboratory cleanliness. Sloppy technique, and a cluttered, dusty laboratory make maintenance of pure cultures very difficult, if not impossible.

1. Disinfectants

The terms disinfectant and antiseptic are used to describe chemicals that kill or prevent the growth and multiplication of microorganisms. These chemicals operate in one or more of the following ways.

a. Coagulate protein
b. Inactivate enzymes
c. Oxidation, usually by breaking down enzymes on the surface of microorganisms
d. Formation of salts with proteins on the surface of microorganisms
e. Hydrolysis
f. Disruption of the cell membrane
g. Modification of the permeability of the cell membrane

There are several commercially available disinfectants suitable for general laboratory use. These may be found in the catalogs of most scientific supply companies. Whatever products are used, it must be kept in mind that they are toxins, and caution should be exercised in their use. The following three disinfectants are adequate for general laboratory

use, and can be made up from chemicals usually found in the laboratory, or purchased from local stores.

Ethyl alcohol (ETOH) at 50%-70% is a good disinfectant, and is quite useful in the pathology laboratory. Instruments may be dipped in it and flamed, or stored in 70% ETOH after sterilization by other means. The following rule of thumb may be used for preparing various percentages of alcohols: a graduated cylinder is filled to the desired percentage level with 95% ETOH, and then distilled water added to the 95% mark. For example, in preparing 70% ETOH, a 100 ml graduate is filled to the 70 ml mark with 95% ETOH, and then distilled water added to the 95 ml mark. Seventy percent ethyl alcohol is used widely in pathology laboratories to swab down table tops and to clean apparatus. Its chief danger is its inflammability. Too often, careless workers have been burned when using alcohol in proximity to an open flame, forgetting that their hands, laboratory coat sleeves, etc., had alcohol on them. It has an advantage, however, in evaporating completely, leaving no toxic residue which might inhibit growth of cultures, or accumulate to harm laboratory personnel. Containers for alcohol should be labeled as to percentage, and marked flammable. This is the disinfectant preferred by the author, but the following disinfectant is preferred by many other workers, and is very effective.

Sodium hypochlorite (ordinary laundry bleach) is probably the most widely used oxidizing agent, and is a safe and quite efficient disinfectant for laboratory use. Its main disadvantages, as this writer sees them, are its corrosive activity (I have seen laboratory table tops finely pitted from repeated use of sodium hypochlorite), and the irritation that may be caused by the small amounts of chlorine gas given off. It is probably the most readily available and inexpensive disinfectant, and is usually sold in grocery and hardware stores at a concentration of 5.25%. For use as a general laboratory disinfectant it should be diluted to 1%-2%. Both stock solutions and containers of diluted sodium hypochlorite should be labeled as to their contents, and marked POISON.

A third general disinfectant that has been widely used in bacteriology and pathology laboratories may be prepared as follows. A stock solution is prepared by dissolving 1 part mercuric chloride in 2.5 parts of commercial (technical grade) hydrochloric acid. Prepare a small bottle of the stock solution (label: POISON) and for use as a disinfectant, add 2.5 ml of the stock solution to one liter of water. This may be tinted with a little dye for easy recognition, and labeled POISON. Although this is a good bacteriostatic agent, the author has reservations about its general use. Residues of mercury compounds tend to accumulate, and pose the problem of contamination in culture media, which would affect the growth of microorganisms under study. Mercury compounds are toxic

for man as well as for other animals, and continued exposure to such compounds poses the problem of toxicity to laboratory personnel.

B. General Equipment

In diagnostic studies of diseased insects the choice of equipment such as dissecting instruments, glassware, etc., will vary with the preference and experience of the individual. However, there are several items, basic and necessary to general diagnostic techniques, and these will be discussed here.

In choosing instruments and apparatus, emphasis should be placed on simplicity, cleanness of design, and general usefulness. The student will find that a few well-designed, well-made items of general use will not only be quite adequate for general diagnostic work, but the work can be conducted more efficiently than with a collection of pieces of equipment that individually have limited use. In short, "keep it simple."

This advice applies as well to all techniques and procedures. Particularly for beginning students, the simpler the apparatus and procedures, the greater the chance of success. When confronted with an apparently complex procedure, it is wise to take the time to analyze it and break it down into simple steps.

1. Instruments

The following basic instruments are necessary for general diagnostic work. (a) Two pair of forceps, one fine, and the other heavier, for dissecting insects, teasing apart fungus mycelium for microscopic examination, and for many other applications. The heavier pair may be curved forceps, which many workers feel are more satisfactory for dissecting work. (b) A pair of dissecting needles, (c) fine scissors, and (d) some straight pins (regular insect pins are suitable) are needed for dissections and working with fungi. (e) A scalpel may be useful in dissections, but this is optional. The student will find that a pair of fine scissors (particularly iridectomy scissors) are much more efficient, and versatile for dissecting insects than the scalpel.

A dissecting dish may be prepared by filling a petri dish about halfway with melted paraffin. The paraffin may be mixed with lamp black or powdered charcoal to give a dark background, which is an aid in differentiating between tissues and organs, etc.

Wire inoculating needles or transfer loops are used for making streak plates, broth, or agar stab cultures depending on the type of culture desired. The loop is made from 24-26 gauge platinum wire fixed to a standard bacteriological wire-loop holder. The wire is formed into a