Israel Journal of Entomology

Vol. XXIII(1989) pp. 17-19

ISOLATION AND IDENTIFICATION OF INSECT PATHOGENS AND CHARACTERIZATION OF THEIR INSECTICIDAL PROPERTIES

B. A. FEDERICI
Division of Biological Control, Department of Entomology,
University of California, Riverside, California 92521 USA

ABSTRACT

Many pathogens of insects, particularly those in which the life cycle includes an obligatory phase in the host, produce obvious signs of disease that permit the type of causative agent to be readily identified, and subsequently isolated and characterized. Some typical examples are the various virus diseases of lepidopterans, rickettsial diseases of most insects, and fungal and protozoan diseases in general. Determination to type, and in many cases a more specific identification, can be made on the basis of the gross pathology caused by the pathogen, and a determination of the cytopathology based on examination of host tissues by phase microscopy. Where such methods do not lead to a definitive identification, the diseased specimen and/or pathogen can be referred to an authority for a more accurate diagnosis and identification.

Bacteria differ from most other pathogens in that, as important as they have become in insect control, they have become in insect control, they only rarely cause obvious disease in field populations under natural conditions. Infection of scarabs by *Bacillus popullae*, where the larvae turn milky white due to the accumulation of sporulated cells in the hemolymph, is an interesting exception, and there are a few others, but strains of the most commonly used bacterial control agent, *Bacillus thuringiensis,* produce no definitive gross signs of disease prior to death. This is largely because *B. thuringiensis* kills insects through the action of toxins, without, in most cases, proliferating throughout the body before the insect dies. Thus, moribund or dead insects in which no cause of disease is obvious are good sources of *B. thuringiensis* isolates, as are frass, detritus from insect habitats, and soil.

Once an isolate *of B. thuringiensis* has been obtained, it can be characterized in regard to several properties. One of the most useful characteristics is the toxicity to various insects, which can really only be determined by bioassay. Where international standards have been developed, the relative toxicity of a new isolate should be determined in comparison to the standard through bioassay. Other useful characteristics include flagellar and crystal serotype, plasmid complement, number and mass of parasporal body proteins, and structure of the parasporal body. These characteristics in combination can provide a definitive identification for isolates of *B. thuringiensis.* The purpose of this article is to provide a broad overview of the principles and methods used to diagnose insect diseases, and isolate and characterize the pathogens that cause their diseases. I will focus my attention on bacterial pathogens, with particular emphasis on defining properties useful to those of us who are primarily interested in the discovery and development of bacteria that can be used to control insect pests and vectors of disease.

The diagnosis of insect diseases is really no different than the diagnosis of diseases of other types of organisms, such as plants and vertebrates. Diagnosis is a combination of specific information and skill. The informational component involves specific facts about the pathogens and pathologies they cause, and can be learned quite readily through training or textbooks; the skill takes practice and

time to develop, but with a little talent and effort, can be acquired without too much difficulty. When developed to a state of art, a brief examination of the gross pathology caused by a pathogen, depending on the diseases, can often lead to a very quick specific identification, particularly if one knows the species of the diseased insect. Gross pathology by itself, or in combination with histopathology, which often can be determined by examination of wet mounts by phase microscopy, will usually enable the trained examiner to diagnose the type of disease. Again, occasionally a specific identification will be possible, depending on the disease. If a specific diagnosis is not possible, a more detailed characterization of the pathogen must be undertaken. Particularly useful texts which provide guidance in regard to appropriate methods include the manuals developed by Poinar and Thomas (1978), Weiser (1982), and the chapters on diagnosis and identification in H.D. Burges' (1981) edited volume entitled "Microbial Control of Insect Pests and Plant Diseases, 1970–80."

When it comes to bacteria, particularly *Bacillus thuringiensis*, the methods of diagnosis differ considerably from those for the diseases caused by viruses, fungi, and protozoa. Many pathogens belonging to the latter groups cause specific color changes in diseased hosts well in advance of death. However, with the bacilli, except for *B. popilliae*, color changes occur, usually a darkening of the body, but generally not until after death. Becilli that kill their hosts via toxins do cause an obvious paralysis, which can occur shortly after intoxication, but this often goes unnoticed, especially under field conditions. As a result, in moribund or dead insects suspected of being diseased, a pathogen such as *B. thuringiensis* is often implicated as possible cause of disease only when no other pathogens are the obvious cause. Fortunately, *B. thuringiensis* is easily isolated from dead insects, where it was a cause of disease, enabling a diagnosis.

Because it is not an obligate pathogen of insects, and can be cultured with ease on a variety of media, *B. thuringiensis* can be isolated from a variety of substrates other than dead insects, including frass and soil. Due to its ubiquity and ease of isolation, we now have well over 1000 recorded isolates of *B. thuringiensis*, a number which will likely increase significantly as a result of isolation programs currently underway.

The availability of these isolates is useful as it is probable that at least some of them, or those yet to be found, will have novel protein toxins. Identification of these toxins enables us to increase the range of insects we can control with *B. thuringiensis*, using either the native proteins or those manipulated or modified through the use of recombinant DNA technology. At present we are confronted with a major problem of how to efficiently screen for, characterize, and catalog new isolates and toxins. In most cases, existing isolates have been screened, if at all, through bioassays against lepidopterous larvae. This was done because for many years these were the only insects known to be susceptible to the protein toxins of *B. thuringiensis*. With the discovery of *B. t. israelensis*, the PG-14 isolate of *B. t. morrisoni*, and more recently, *B. t. tenebrionis*, isolates are now screened against representative species of insects of the orders Lepidoptera, Diptera, and Coleoptera. Ideally, a much greater range of insects from a broader range of orders should be screened, though this is costly and time-consuming. However, now that several venture capital firms and established chemical companies have entered this arena, it will likely be done.

Bioassay offers the only true test of toxicity to an insect. However, other methods can be used to identify isolates of potential interest. In my laboratory, we look for unusual parasporal body shapes. Once such an isolate is identified, we purify the parasporal bodies, characterize the protein complement via polyacrylamide gel electrophoresis, and determine the number of inclusions within the parasporal body using electron microscopy. If we find unique proteins, we also determine the number and size of the plasmids in the isolate as part of our characterization, have the isolate serotyped if the serotype has not been determined, and carry out bioassays against a selected range of insects. Using this strategy, we have found three unique isolates out of twelve we selected for examination from Dr. Howard Dulmage's collection.

To close, I would like to comment on methods of cataloging isolates of *B. thuringiensis*. On

occasion recently, the serotyping of isolates has been ignored, and instead biochemical procedures which have little, if any, relationship to toxicity, have been used by some to identify and name more than 50 new varieties of *B. thuringiensis*. Serotyping is far from perfect, but it has enabled us to proceed with some sense of order over the past twenty years. The serotyping system should be used as a method of cataloging isolates, if for no other reason, until a better system is found for cataloging that also provides insight to the toxicological properties of an isolate. In addition to serotyping, other features useful in the characterization of an isolate are the parasporal body protein complement, plasmid complement, and, of course, host range and level of toxicity.

REFERENCES

Burgess, H.D. 1981. Microbial Control of Pests and Plant Diseases. Academic Press, New York and London.

Poinar, G.O. and Thomas, G.M. 1978. Diagnostic Manual for the Identification of Insect Pathogens. Plenum, New York.

Welser, J. 1982. Guide to the Field Determination of Major Groups of Pathogens Affecting Arthropod Vectors of Human Diseases. WHO/VBC/82.860 World Health Organization, Geneva, Switzerland.