Diagnostic Service identifies insect pathogens

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The Department of Entomological Sciences at the University of California in Berkeley offers the only diagnostic service in the United States with equipment and experienced personnel for formal laboratory diagnosis of insect diseases. The Diagnostic Service, started in Berkeley by Edward A. Steinhaus in 1944 as an aid to university entomologists, rapidly grew into a worldwide service. The Service was run by Professor Steinhaus and Gordon A. Marsh until 1964, and has been run by the present authors since then (fig. 1).

Since 1944, the Diagnostic Service has received and processed 3560 accessions from all parts of the globe, mostly submitted by researchers in federal, state, or private institutions, but also from amateur entomologists, students, farmers, bee keepers.

The Diagnostic Service:

- Identifies pathogens causing insect diseases.
- Discovers new pathogens, particularly those that are potential biological control agents against crop pests or vectors of plant, animal, and human diseases.
- Assists in controlling diseases affecting beneficial insects.
- Gathers information on the host range and distribution of various pathogens.
- Maintains a reference collection of insect pathogenic microorganisms.

Identifying insect pathogens

The diagnosticians keep abreast of descriptions of new insect pathogens in all five major groups: fungi, bacteria,

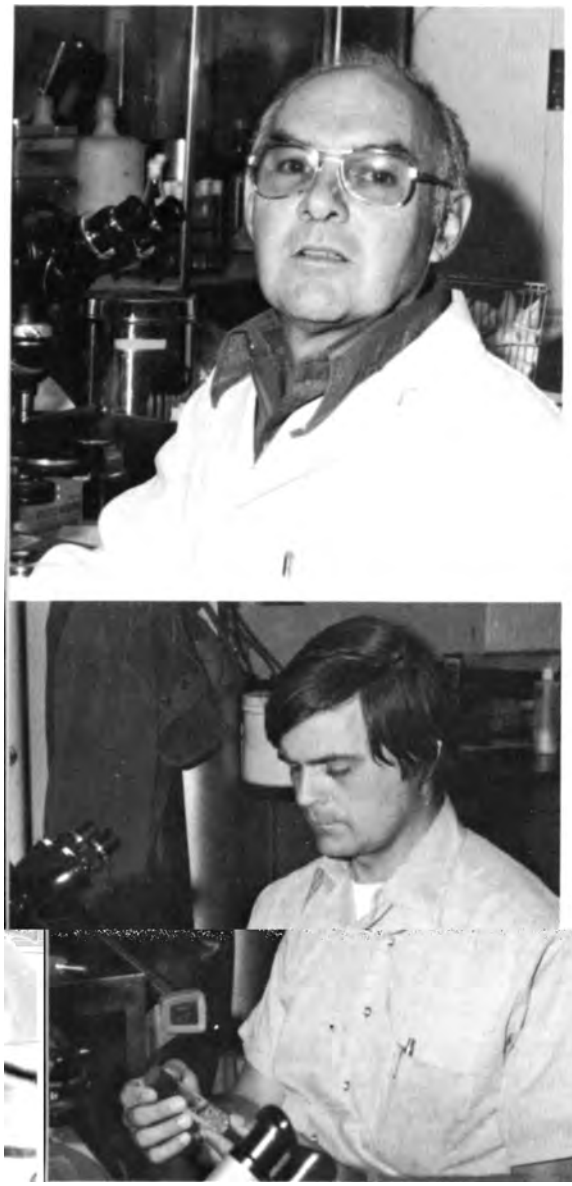

Fig. 1. Insect pathologists responsible for the Diagnostic Service: *top*, Gerard Thomas; *bottom*, George O. Poinar, Jr.

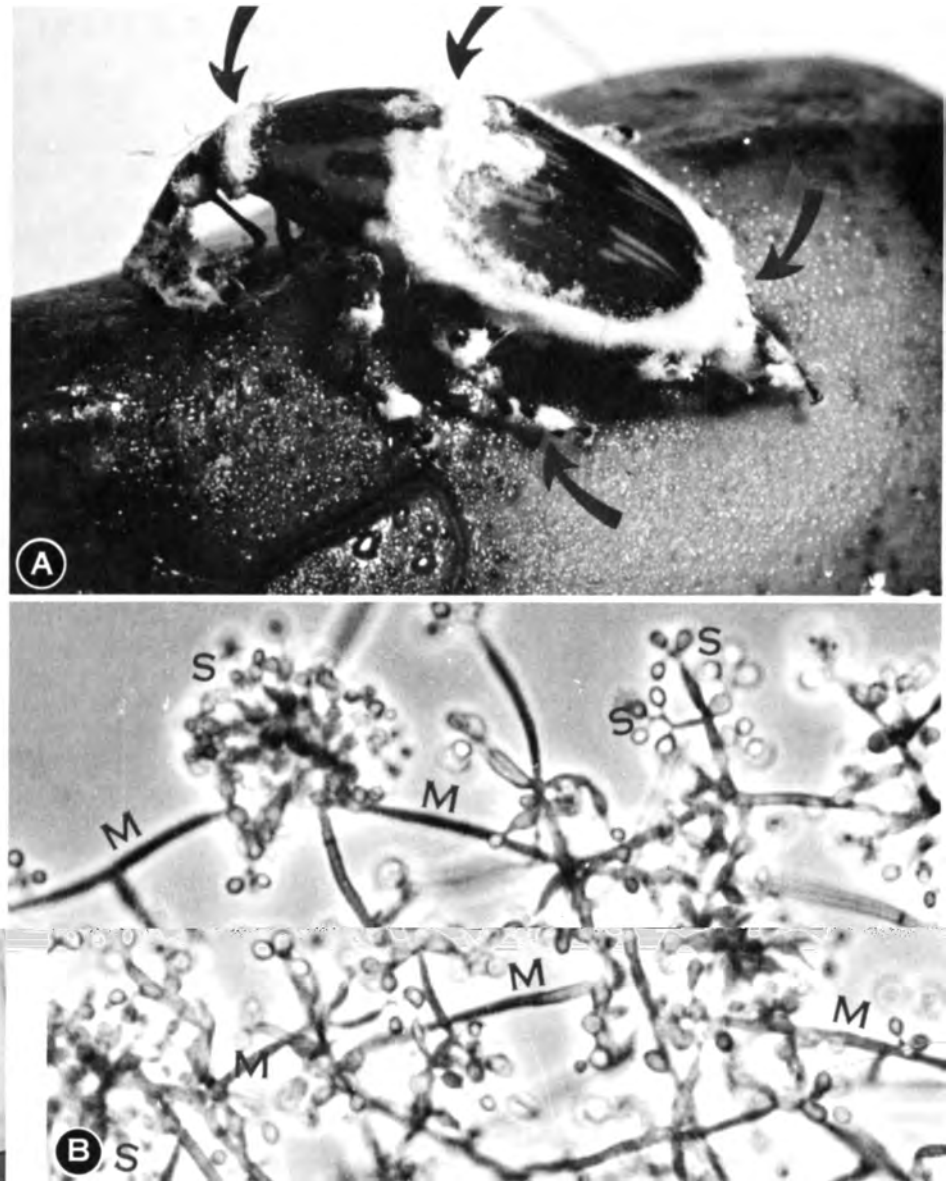

Fig. 2. *A*. Adult weevil infected with the fungus *Beauveria bassiana*. Note the fungal mycelium emerging from intersegmental areas (arrows). *B*. Photomicrograph showing the mycelium (M) and spores (S) of *Beauveria bassiana*.

Fig. 3. Electron microscopist Roberta Hess often uses the electron microscope to confirm suspected virus infections.

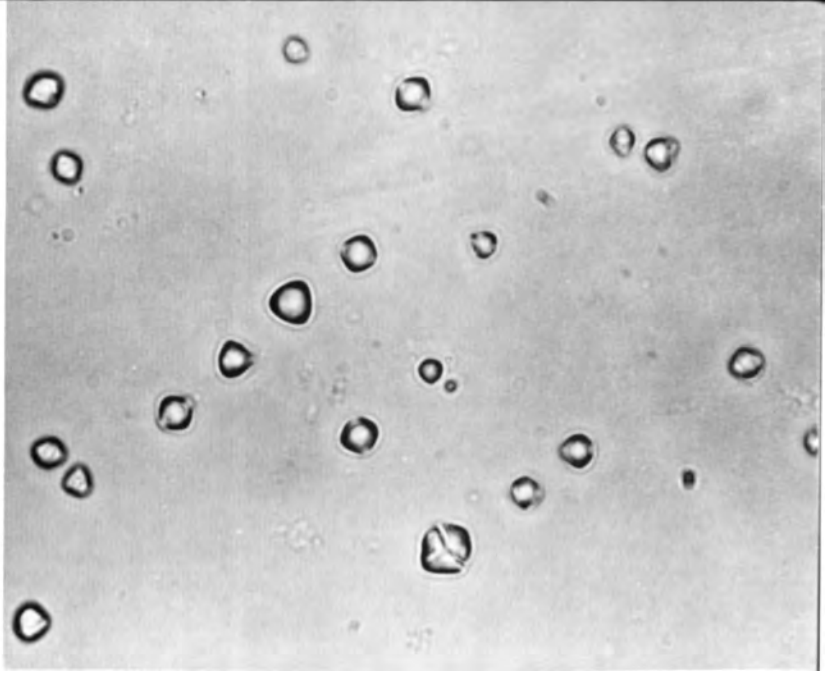

Fig. 4. Photomicrograph of blood from a hemlock looper infected with a nuclear polyhedrosis virus showing the polyhedral inclusion bodies which contain the virus particles.

protozoa, viruses, and nematodes. Fungi are by far the most common group of pathogens received for diagnosis, probably because they are easily observed on the outside of insects (fig. 2). At the opposite extreme, viruses can be positively identified only with the aid of the electron microscope (fig. 3) or highest power of the light microscope (fig. 4).

Nematodes are sometimes fairly large, and can be easily seen through the cuticle of some insects (fig. 5). The common insect pathogenic bacteria can usually be identified with a few simple laboratory tests after being cultured on nutrient plates (fig. 6). Protozoan pathogens generally cannot be grown on artificial media, and must be identified by spore size, shape, structure, and formation (fig. 7).

Discovering new pathogens

Studies of the many new pathogens discovered by the Service have led to a better understanding of the dynamics of natural outbreaks of disease, and to the development of techniques for microbial control.

In the first reported instance of successful microbial control arising from work done in the Diagnostic Service, a polyhedrosis viral disease was identified on an alfalfa caterpillar. This lethal disease usually appears too late in the larval life of the insect to prevent damage to the alfalfa.

Field trials in 1948, extended in 1949 by using airplanes to spread the polyhedrosis virus over fields infested with the alfalfa caterpillar, showed that epidemics of the polyhedrosis virus could be started artificially, advancing the time of outbreak of the disease and reducing insect populations to levels below those at which they could inflict economically important crop damage.

Professor Steinhaus recognized the potential of *Bacillus thuringiensis* (B.t.), a spore-forming bacterium which had been isolated from a meal moth in Germany before World War II, as a control agent against a number of California crop pests. Through cooperative studies with farmers and scientists at the university and in private industry over a period of years, *Bacillus thuringiensis* was developed into the first microbial insecticide, and is now licensed by the FDA for use against insect pests of a number of important crops, including cotton, corn, alfalfa, artichokes, grapes, fruits, and nuts.

In 1966, specimens of diseased codling moth larvae brought in by a univer-

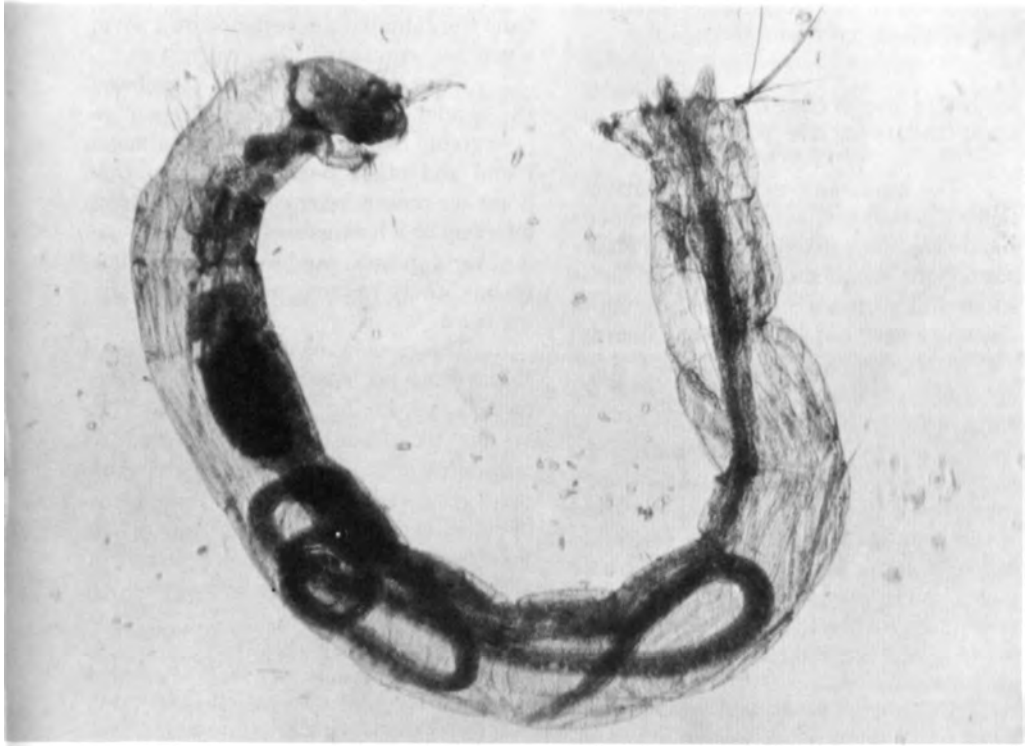

Fig. 5. A midge larva (*Tanytarsus* sp. Chironomidae) infected with the mermithid nematode, *Hydromermis conopophaga*.

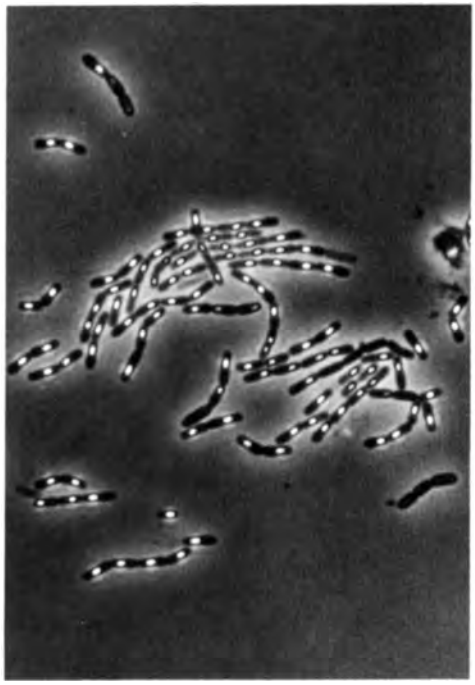

Fig. 6. Phase contrast photomicrograph of *Bacillus cereus*, a common insect pathogen. The bright object in the bacterial cells is the infective spore.

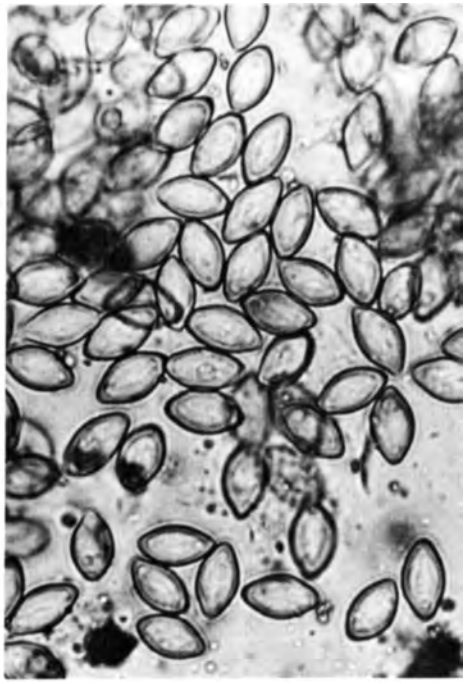

Fig. 7. Photomicrograph of spores of an insect pathogenic protozoan, *Mattesia* sp., from the potato tuberworm.

sity insect pathologist were diagnosed as suffering from a granulosis virus infection. This scientist, in cooperation with farmers, is seeking FDA approval for the use of this virus as a microbial control agent against codling moth.

Curing diseases of beneficial insects

In crowded laboratory or insectary colonies of insects, disease can spread rapidly, decimating stocks of insects intended for testing insecticides, raising parasites, and genetic studies. For example, the Diagnostic Service determined that insects used by a California chemical company in testing a new insecticide against the variegated cutworm were being killed by a granulosis virus not previously reported in the United States. The Service both advised on methods for rearing healthy insects and explored the possibility of a new microbial control agent.

In 1968 "chalk brood," an unusual disease of honey bees, was diagnosed from mummified larvae sent to the Diagnostic Service by the California Chief Apiary Inspector. Since this first report of chalk brood in the United States, it has also been diagnosed in alfalfa leaf cutter bees being reared in domiciles for alfalfa pollination. Researchers at UC Berkeley and Davis, bee keepers, and alfalfa growers are cooperating to find methods of controlling this disease.

The Diagnostic Service often renders assistance to state and federal agencies by checking for disease in shipments of insects imported from foreign countries to be reared for use in biological control of noxious weeds or pest insects.

The culture collection

The Diagnostic Service maintains a viable culture collection of insect pathogenic viruses, bacteria, fungi, and some nematodes. Most, except nematodes, are maintained in lyophilized (freeze-dried) form. A list of microorganisms currently maintained in the collection is available upon request.

How to submit diseased specimens for diagnosis

For quick and complete identification of pathogens, submitted specimens should contain relatively pure and viable pathogenic organisms. Diagnosis is most successful on fresh specimens; timing is therefore very important. When quarantine restrictions prohibit shipment of living diseased insects, freshly dead specimens are adequate.

One of the biggest problems in shipments from long distances, and especially from warm climates, is that as diseased specimens age, the organism originally responsible for the condition may break down, and the action of secondary agents may mask the original etiology. Specimens should be submitted before general deterioration occurs.

If this can't be done, it is sometimes helpful to submit a few additional specimens in a preservative, such as 70 percent alcohol or 5 percent formalin. In most cases it is better not to use preservatives, as the identification of bacteria and fungi (the two most common types of disease organisms encountered) depends on tests requiring their growth on various nutrient media.

Enclose specimens in a clean dry vial, pill box, or other suitable container, and include the following information whenever possible.

- Common and scientific name (including the family and author of the species) of the insect
- Date and locality of the collection
- Conditions under which the disease material was found and the extent of the outbreak
- Any abnormal behavior or external signs or symptoms of the diseased specimens
- Occurrence of other agents or factors (insecticides, abnormal temperatures or humidity, crowding) that might account for the condition

All shipments should be labeled "Dead biological specimens of no commercial value" and sent to: The Diagnostic Service, Department of Entomological Sciences, University of California, Berkeley CA 94720. Please include the fee of $5 per accession. An accession is defined as a group of specimens of a single insect species received at one time.

Each shipment is acknowledged, and the sender is notified of the assigned accession number. Information received from the submitter is retained on a permanent accession card.

When the diagnosis is completed, the sender will receive a diagnostic report giving him the name of any pathogen found and other pertinent information. Some diagnoses, such as a microsporidian infection or a bacterioses caused by *Bacillus thuringienses*, can be completed quickly, but others require more time-consuming tests.

The sender may submit additional material or is free to make any other inquiry regarding the diagnosis. The sender is entitled to use the information in publications. The Entomology Department reserves the right to include the results of the examination in any of its publications and to use the diseased insects or isolated pathogens in any way it may desire.

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