I.5 Identification of Fungal Pathogens of Grasshoppers

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Introduction

Grasshoppers are host to a wide range of micro-organisms that cause disease. Of these, the fungi provide spectacular appearance of disease symptoms. On a larger scale, fungi can devastate whole populations of grasshoppers. Some of these fungi cannot grow without a grasshopper host (obligate pathogens); other fungi are easily cultured in the laboratory and can infect a wide range of insects including grasshoppers (facultative pathogens). In this chapter, we will examine methods used to discriminate pathogenic fungal infections from bacterial or nonpathogenic fungal growth on a dead insect. We will also discuss the most probable fungal infections found in the field.

Fungi Pathogenic to Grasshoppers

There are two main groups of fungi that have species pathogenic to grasshoppers: the zygomycetes and the deuteromycetes. Some zygomycete species are obligate pathogens of grasshoppers. The deuteromycetes that are pathogenic to grasshoppers are facultative pathogens.

Zygomycetes (Entomophthorales).—The pathogenic Entomophthorales are complex and poorly understood. The only confirmed pathogens that infect grasshoppers belong to the *Entomophaga grylli* complex. There are at least three pathotypes of the *E. grylli* complex. The term pathotype refers to the type of grasshopper that is infected. The three pathotypes also differ with respect to their life cycles, host grasshoppers, and growth requirements (Ramoska et al. 1988). Two of the pathotypes are native to North America, and a third pathotype has been isolated from a grasshopper species in Australia.

Pathotype 1 infects the bandedwinged grasshoppers (Oedipodinae). The grasshopper species most commonly infected are *Camnula pellucida* and *Dissosteira carolina*. Pathotype 2 infects melanopline grasshoppers (Melanoplinae) and the species most commonly infected are *Melanoplus* and *Hesperotettix* spp. Pathotype 3, the Australian isolate, infects bandedwinged and melanopline grasshoppers under laboratory conditions.

Disease Characteristics.—*Entomophaga* spp. are the most common and widespread pathogens of grasshoppers in North America. Disease symptoms in the advanced stage are characteristic and easy to recognize. Shortly before death, infected grasshoppers crawl to the tops of plants, fenceposts, or any other elevated position. There they die with their legs wrapped around the plant stalk and heads pointed upward.

Examining the specimen found in the characteristic "summit disease" is simple. Open the abdomen or poke a hole in it with a sterile toothpick and a place sample of this on a microscope slide with a drop of water. The inside of the grasshopper may contain a variety of fungal bodies, but the most common are large (50 m in diameter), spherical, thick-walled resting spores. If the grasshopper is *Camnula,* the infection is probably pathotype 1; in a melanopline grasshopper, probably pathotype 2.

External sporulation is also used to discriminate between pathotype 1 and 2 infections. Grasshoppers suspect of *E. grylli* infection are placed in a humid environment, such as petri dish containing 1.5 percent agar. Within 24 hours some of the specimens will show sporulation (white rings) on the abdominal segments. Pathotype 1 will show external sporulation (conidia approximately 50 μm in diameter) as well as the internal resting spores. Pathotype 2 will not show external sporulation.

North Dakota Introductions.—Recently, pathotype 3 *(E. praxibuli)* has been introduced into North Dakota from Australia. This fungus infects both oedipodine and melanopline grasshoppers. External growth on a melanopline grasshopper may be indicative of *E. praxibuli* infection. However, we caution against the use of morphology and growth characteristics as tools in differentiating the three *Entomophaga* pathotypes.

We have developed DNA (deoxyribonculeic acid) probes that could be used differentiate the three pathotypes (Bidochka et al., 1995). We have also devised a method by which the resting spores of these fungi can be fractured, and the DNA can be isolated and used as a template for the pathotype-specific probes.

Deuteromycetes.—Worldwide, the most common deuteromycete infections in grasshoppers are *Beauveria bassiana, Metarhizium anisopliae,* and *Aspergillus flavus.* In central Africa, *Metarhizium flavoviride* is found more commonly than *M. anisopliae.*

Disease Characteristics.—Grasshoppers that have an external white or green mycelial (filamentlike fungus) growth are also potential suspects of fungal infection. The most common non-*Entomophaga* infections found in the field are *B. bassiana, M. anisopliae* and *A. flavus.* *B. bassiana* infection is characterized by white mycelial growth on parts of the surface of the grasshopper; *M. anisopliae* and *A. flavus* infections are characterized by green surface growth. The conidia of these fungi are much smaller (approximately 5–10 μm in diameter) than the conidia of *Entomophaga grylli. M. anisopliae* conidia are rod shaped, but *M. flavoviride* conidia are more rounded or elliptical. *B. bassiana* conidia are globose (round or globelike), and *A. flavus* conidia are spherical. For more detailed descriptions and microphotographs of entomopathogenic fungi, refer to Samson et al. (1988) and Poinar and Thomas (1984).

Isolating Pathogenic Deuteromycetes.—Several selective media for the isolation of *B. bassiana* and *M. anisopliae* have been tested. The best medium for selective isolation of *B. bassiana* and *M. anisopliae* is 30 g of wheat germ in 1 L of water, autoclaved for 10 minutes and filtered through four layers of cheesecloth. To this are added 0.25 g chloramphenicol, 0.75 mg benlate (50 percent benomyl), 0.30 g dodine, 10 mg crystal violet, and 15 g agar (Chase et al. 1986).

The mycelia on the surface of the grasshopper can be picked with a sterile toothpick or sterile wire loop and streaked onto this agar-medium. The petri dishes should be wrapped in aluminum foil because exposure to light delays colony growth. Optimal growth occurs at 79 °F (27 °C) for these fungi. If the fungus grows, then it may be one of the pathogenic deuteromycetes. If the fungus does not grow, it may simply be a nonpathogenic fungus growing on the dead grasshopper.

B. bassiana, M. anisopliae, and *M. flavoviride* also can be differentiated based on patterns of DNA fragments generated by random amplification of polymorphic DNA (RAPD) and with molecular probes using the RAPD fragments (Bidochka et al. 1994).

Other fungi that may infect grasshoppers include *Verticillium lecanii, Nomuraea rileyi,* and *Paecilomyces* sp.

Assessment of Fungal Disease

To prove that a certain fungal isolate is the causative agent in grasshopper death, lab personnel must follow these steps: (1) The fungus must be isolated from the grasshopper. (2) The fungus must be grown in media. (3) The fungus must cause disease either by injection of conidia into the body cavity or by exposing the insect to fungal conidia. Most entomopathogenic fungi normally infect by passing through the insect exoskeleton. It is preferable that the host insect from which the fungus was isolated be the test insect. This is particularly true for the Entomophthorales. For deuteromycetous fungi, a test insect such as wax moth larvae *(Galleria mellonella)* or silkworm larvae *(Bombyx mori)* may be used. (4) Finally, the fungus must be reisolated from the test insect.

The best diagnostic tools for differentiating *B. bassiana, M. anisopliae, M. flavoviride,* and the *Entomophaga* are molecular probes. The use of these probes is not difficult, and results are generally conclusive. In the near future, the use of such probes will be commonplace in fungal taxonomy.

Suggested References

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