Paenibacillus popilliae

(Eubacteriales: Bacillaceae)

formerly Bacillus popilliae

Milky Disease

Healthy beetle larva (Top); larva infected with B. popilliae (Bottom).

Photo: Michael Klein, USDA, ARS, Horticultural Insects Research Lab, OARDC, Wooster, OH

The Japanese beetle, *Popillia japonica*, was accidentally introduced into the USA early this century. Although it is not a problem in its area of origin, the beetle causes serious damage in the USA. It spread rapidly from the initial sightings in New Jersey (1916) and today it is found over roughly half of the country in almost every state east of the Mississippi. It is a problem as an adult beetle because it feeds on a wide range of ornamental and crop plants, eating the tissues between the veins, and it accumulates on ripening fruit, causing substantial damage. It is also a problem in the larval stage because the adult beetles lay their eggs in turf and the grubs destroy the grass roots.

By the 1930s, the infestation had become so extensive that a search for a control measure was undertaken which led to the discovery in nature of some diseased larvae. Milky spore bacteria were isolated. The term "milky disease" comes from the larva's pure white appearance when infected with *B. popilliae*. *B. popilliae* was the first insect pathogen to be registered in the U.S. as a microbial control agent.

Appearance

P. popilliae is a Gram-positive spore-forming rod of1.3 to 5.2 x 0.5 to 0.8 micrometers.

Habitat

A fastidious organism, *P. popilliae* grows only on rich media containing yeast extract, casein hydrolysate, or an equivalent amino acid source, and sugars. Several amino acids are known to be required for growth, as well as the vitamins thiamine and barbituric acid. Trehalose, the sugar found in insect hemolymph, is a favored carbon source, although glucose can also be used.

Japanese beetle adult.

Photo: Mindy Proscia

Pests attacked

Japanese beetle is the exclusive host of the strain of *P. popilliae* which is sold commercially. However, other *P. popilliae* strains (and *P. lentimorbus*, which is considered a strain of *P. popilliae* by some experts) have other scarab hosts and are specific to different beetles in the family Scarabaeidae, which includes the Japanese beetle and the chafers - important pasture pests, but also the beneficial dung beetles.

Spores which reside in the soil and have been ingested by beetle larvae germinate in the larva's gut within 2 days and the vegetative cells proliferate, attaining maximum numbers within 3 to 5 days. By this time, some of the cells have penetrated the gut wall and have begun to grow in the hemolymph, where large numbers of cells develop by day 5 to 10. A few spores also are formed at this stage, but the main phase of sporulation occurs later and is completed by 14 to 21 days when the larva develops the typical milky appearance.

In laboratory conditions, the larva remains alive until this stage and usually contains about 5 x 109 spores. In field conditions, however, there are reports that larvae sometimes die earlier, before the main phase of sporulation is completed. This is of concern because sporulation stops when the host dies and the larva ultimately releases fewer spores to maintain the level of infestation of a site.

Relative effectiveness

The advantages of using commercial preparations of *P. popilliae* include the very narrow host range (they are effective against Japanese beetles, only), their complete safety for man and other vertebrates, their compatibility with other control agents including chemical insecticides and insect-pathogenic nematodes, and their persistence.

The disadvantages include the high cost of production *in vivo*, the slow rate of action, the lack of effect on adult Japanese beetles, which also cause obvious and distressing damage, and the need for large areas to be treated for effect (see below). The narrow host range, which is environmentally very desirable, is also a disadvantage: managers must accurately identify the infesting grub species to determine if it is Japanese beetle. If there are other grubs present, they will not be attacked.

The treatment is most effective when applied on a region- or state-wide basis (or at least to relatively large areas) to reduce overall the levels of beetle infestation. It is less appropriate for use by small landowners, who may control the larvae in their own turf only to find their trees and shrubs being eaten by beetles from their neighbors' properties. Also, because *P. popilliae* is obligately dependent on its hosts for sporulation and because some larvae may not ingest spores (or not ingest enough to cause disease) a periodic resurgence and decline of the pest problem can be expected. The success of the control program must be judged not on this basis but by the fact that over a number of years the mean level of pest damage is lower than it would be in the absence of *P. popilliae*.

Between 1939 and 1953, over 100 tons of spore powder was applied to turf in over 160,000 sites in the U.S. as part of a government program. Larval numbers in the turf decreased 10- to 20-fold and the population stabilized at this new low level with corresponding reductions in the levels of adult beetle damage.

Recent research indicates that in some regions of the U.S., *P. popilliae* appears to be losing its virulence against Japanese beetles. Only 0.2% of larvae collected from field sites showed symptoms of milky disease compared to 1946 with 41.5% disease incidence. Also, a recent field study in Kentucky showed that commercial formulations of *P. popilliae* were only moderately infective (39-44%), that infected grubs consumed the same amount of roots as uninfected grubs, and that lower grub populations could not be linked to infection. Researchers concluded that earlier reports of success were limited to very high infestations of grubs where other stresses may have increased their susceptibility to diseases.

The cause of death in insects infected with *P. popilliae* is not fully known. Physiological starvation caused by the growth of bacterial cells in the hemolymph seems the most likely explanation, and fat reserves of diseased larvae have been shown to be much reduced compared with those of healthy larvae. However, toxins also may be involved because they have been detected in culture filtrates of the bacteria and shown to be lethal on injection. Recently, a crystal protein from sporulating cells of *P. popilliae* was found to have similarities to one of the Cry toxins of *B. thuringiensis*. This protein might contribute to pathogenic invasion through the gut wall.

Conservation

For general information about conservation of natural enemies, see Conservation in the Tutorial section on this site

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Commercial Availability

Readily available.

Acknowledgement

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Taken from:

Deacon, J.W. 1998. Profiles of Microorganisms - Biological Control: *Paenibacillus popilliae*. Prepared for the course, Microbiology 3m, Biological Teaching Organisation, University of Edinburgh. URL: http://www.biology.ed.ac.uk/research/groups/jdeacon/microbes/control.htm

Additional references

Redmond, C.T. and D.A. Potter. 1995. Lack of efficacy of in-vivo and putatively in-vitro produced *Paenibacillus popillia*e against field populations of Japanese beetle (Coleoptera: Scarabaeidae) grubs in Kentucky. J. Econ. Entomol. 88: 846-854.

Zhang, J. Hodgman, T. C., Krieger, L., Schnetter, W., and H.U. Schairer. 1997. Cloning and analysis of the first cry gene from *Paenibacillus popilliae*.J. of Bacteriology. 179: 4336-4341.