Multiplying Metarhizium for better biopesticides

By Jan Suszkiw, Agricultural Research Service – USDA

Metarhizium anisopliae is one mould you might not mind having around. Instead of growing on bread or shower curtains, it prefers the bodies of ticks and insect pests, such as termites, locusts, tsetse flies, and others.

Indeed, a Metarhizium strain dubbed 'F52' is the chief active ingredient in mycoinsecticide products for controlling soft-bodied ticks and certain beetles and weevils.

Now, even better mycoinsecticides – targeting soil-dwelling insect pests – could be on tap, thanks to ARS scientists' discovery that Metarhizium can produce specialised clumps of fungal cells called 'microsclerotia'.

Agricultural Research Service microbiologist Mark A. Jackson and ARS entomologist Stefan Jaronski made the discovery in 2004 and have since developed a patent-pending method of churning out billions of microsclerotia inside vats called fermentors.

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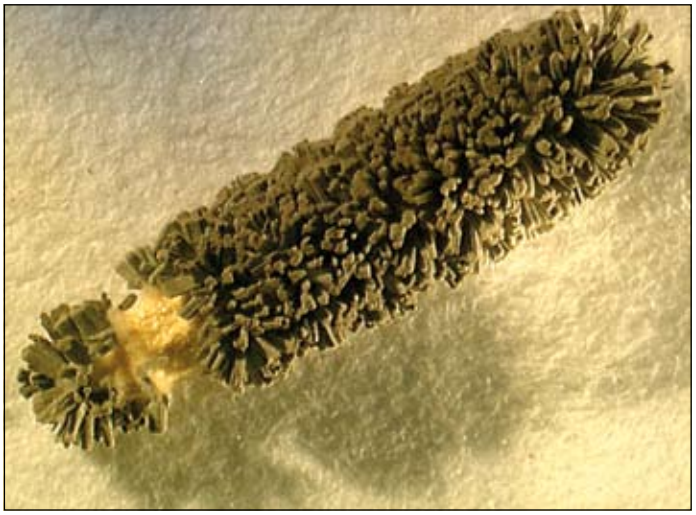
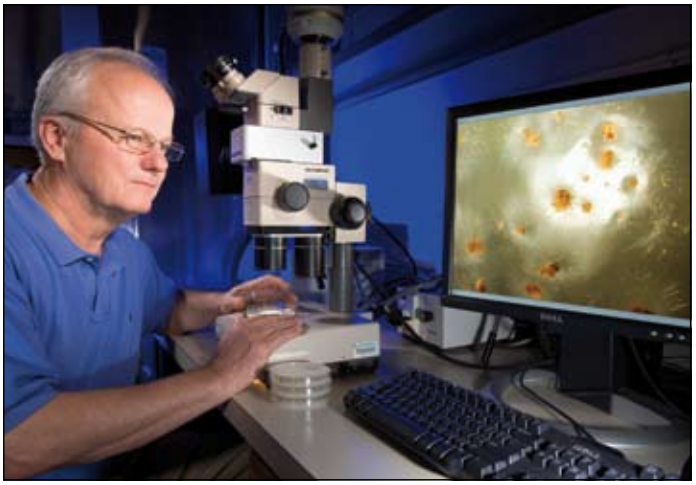
Healthy sugarbeet root maggots (about 6 mm long), fat from feeding on and severely damaging a sugar beet root. In this stage, they will overwinter to pupate and emerge the following spring. (PHOTO: Stefan Jaronski)



The fungus *Metarhizium anisopliae* sporulating on a sugarbeet root maggot. (PHOTO: Stefan Jaronski)



Microbiologist Mark Mark evaluates spore production by air-dried microsclerotia (rewetted and incubated on water agar here) produced by *Metarhizium anisopliae*. (PHOTO: Peggy Greb)

Making more and tougher fungi

Before the duo's finding, only plant-disease-causing fungi like *Sclerotinia sclerotiorum* were reported to produce microsclerotia – not their insect-infecting brethren.

"We found with Metarhizium that we could produce these sclerotial bodies in liquid culture under certain conditions," says Mark, with ARS's National Center for Agricultural Utilization Research in Peoria, Illinois. "The advantage of this is that we can now make a form of this fungus that can survive drying and storage for easy application by farmers to the soil to kill insects."

Traditionally, the form of choice for making mycoinsecticides has been the conidia, or spore, which forms thin tubes that penetrate an insect host's outer shell, or cuticle. The fungus only infects certain insect hosts, however, and never people, pets, or livestock.

"Metarhizium's conidia are like little time bombs," explains Stefan, with ARS's Northern Plains Agricultural Research Laboratory in Sidney, Montana. "They don't germinate until they contact the insect cuticle. Then, they use a combination of mechanical pressure and a cocktail of enzymes to breach the cuticle and invade the insect's circulatory system. The infected insect invariably dies within a few days."

In a standard production approach, Metarhizium is grown on nutritious cakes called "solid substrate". The fungus produces abundant conidia, which are then collected, dried, and coated onto granules made of corn grits or other granular carriers or mixed directly into the soil. But the solid-substrate approach is time consuming and labour intensive for this purpose, notes Mark.

Microsclerotia – tight bundles of pigmented fibres that resemble pepper flakes – are a far tougher form of fungus. Moreover, they serve as a safe haven on which Metarhizium's conidia can be readily produced to infect insects that get too close while crawling about in the soil.

Other researchers have produced granules from air-dried, regular mycelium (the fungus's main body) or mycelium encapsulated in a polymer, says Stefan. But these forms suffer from poor shelf life or cost too much for most farmers.

ARS scientists have developed a method for producing microsclerotia of *Metarhizium anisopliae*, a fungus that may help combat soil-dwelling pests. Here, technician Angela Payne adds nutrients to a 100-litre fermentor in preparation for a microsclerotia production run using *M. anisopliae*. (PHOTO: Peggy Greb)

Cheaper and faster, too

In studies at Sidney using Metarhizium strain F52, conidia-only granules germinated 7–10 days after being applied to soil. Microsclerotia-based formulations germinated within four days and produced greater numbers of spores.

Mark partly credits the microsclerotia's increased germination rate to their ability to tolerate lower soil moisture. Another factor may be the sheer number of microsclerotia that can be produced and applied to the soil using the liquid-culture technique he and Stefan developed.

The researchers' current microsclerotia production rate is 30 grams of wet fungal biomass (fermentation material containing fungal cells) per liter in about four days. Solid-substrate systems, by comparison, take two weeks to produce commercial quantities of conidia, and more time is needed to prepare the granules.

Microsclerotia can also be formulated into granules and sized more easily than conidia-based formulations. This makes microsclerotia more compatible with farmers' seed planters and pesticide granule applicators. Biopesticide makers stand to benefit, too: "Using microsclerotia should allow companies that make mycoinsecticides to get into markets where, ostensibly, the size and shape of their products have kept them out," Stefan says. Microsclerotial granules should also readily qualify for the organic crop market, whereas the binders used with conventional granular carriers disqualify those granules, he adds.

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