Fungus-Based Insecticides

Fungi and insects are among the most abundant groups of organisms on the planet. Fungi and insects interact in a multitude of ways, ranging from simple coexistence to symbiosis, to predator/prey relationships in both directions.

Fungi play several roles in nature, and an important one is being decomposers, or detritovores. So, of course, they consume dead insects as they do dead other things. In order to recruit a fungus to serve as an insecticide, that fungus would have to be able to attack and kill living insects. Over a thousand known fungal species can do that.

Organisms that are able to cause disease and/or death in insects are called entomopathogenic. There are entomopathogens among bacteria, viruses, and protozoa as well as fungi. Bt, *Bacillus thuringiensis*, is an example of an entomopathogenic bacterium.

Why seek out mycoinsecticides?

Most gardeners are likely to turn to chemical insecticides when facing a plague of aphids, thrips, etc. And many homeowners turn to chemicals when facing bedbugs, termites, houseflies, ants, fleas and ticks.

The drawbacks of chemical insecticides are several. Many are toxic to humans, and require protective equipment and special conditions for application. And some persist in the environment long after they have fulfilled their purpose or been banned.

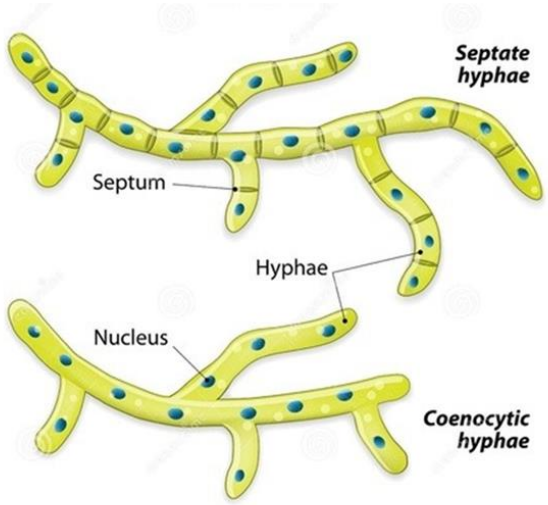
Also, after widespread use, chemical insecticides select for resistance mutations among their insect targets. Thus, for example, control of malaria mosquitos has become difficult as the mosquitos become resistant to one chemical insecticide after another.

Chemical insecticides tend to be non-specific, so beneficial insects will be killed along with the pests.

In principle, fungal insecticides avoid most of these problems. They are not harmful to humans, and are biodegradable organic matter. The acquisition of resistance to mycoinsecticides is difficult, because predator and prey are both evolving. Specificity may, however, be an issue with some mycoinsecticides.

Fungal life involves hyphae, mycelia, asexual spores, and sexual spores.

Hypha (plural – hyphae): One of the long, slender chains of cells that develop from germinated spores and form the structural parts of the body of a fungus. Hyphae have an average diameter of 4 to 6 μm. The average diameter of human hair is about 50 μm.



Mycelium: The fungal mass formed by growing, branching hyphae.

Conidium (plural – conidia): Asexual reproductive spores or mitospores. They are single cells formed from some hyphal cells. Each conidium can "sprout" to give rise to a new hypha.

Spores: Some fungi have "mating types" and produce fruiting bodies containing sexual spores or meiospores.

Mitospores and meiospores are both often referred to as just "spores."

The exoskeleton of an insect looks like a suit of armor, but has to allow for movement. The insect has hard mineralized chitin plates over much of its body, but the joints between the body segments are of a leathery form of chitin.

Fungi “eat” by secreting digestive enzymes onto their food and absorbing the dissolved nutrients.

They have an array of digestive enzymes capable of penetrating the exoskeleton.

When a sticky fungal spore adheres to a prey insect, the hypha forms quickly and uses digestive enzymes and pressure to drill a tiny hole through the exoskeleton.

The hypha then grows through the hole into the insect's body, where it continues to grow and digest. Eventually it grows throughout the body, leaving an exoskeleton filled with fungus.

When a fungus has fully exploited a food source such as an insect, the next step is to reproduce, so the remaining exoskeleton may be covered with a powdery coating of conidia (muscardine disease), or a fruiting body (mushroom) may form, depending on the fungus.

Ophiocordyceps unilateralis

O. unilateralis is actually several different subspecies, each preying on a different species of ant. Thus the potential would seem to exist to use one of these species to develop a species-specific insecticide.

O. unilateralis is known for infecting warm area carpenter or leaf cutter ants, during which it alters the behavior of the ants in such a way as to maximize the propagation of the fungus. The prey ants often live in an arboreal nest but forage over extensive areas, sometimes having to move on the ground to another tree. This exposes the ant to the possibility of meeting a fungal spore and becoming infected.

An infected ant climbs up into the colony's nest. Within two or three days the infection causes the ant to convulse and fall out of the tree. Then the ant begins to display other behaviors that are never seen in uninfected ants.

The "ant" climbs up an understory plant to a height of about 1 foot, clamps its jaws onto the midrib or other large vessel of a leaf, and hangs there while being consumed from within. It takes about 5 days from infection for the ant to die, and then a fungal fruiting body grows out from the back of the ant's head. Then spores are released over about a square meter of ground.

How to make a zombie ant

Ophiocordyceps unilateralis, a fungus found in the tropical rainforests of Thailand, survives by controlling carpenter ants.

1. INFECTION

A foraging carpenter ant walks through an area of the rainforest floor infested with microscopic spores dropped by a mature fungus. The spore excretes an enzyme that eats through the ant's exterior shell.

2. DEATH GRIP

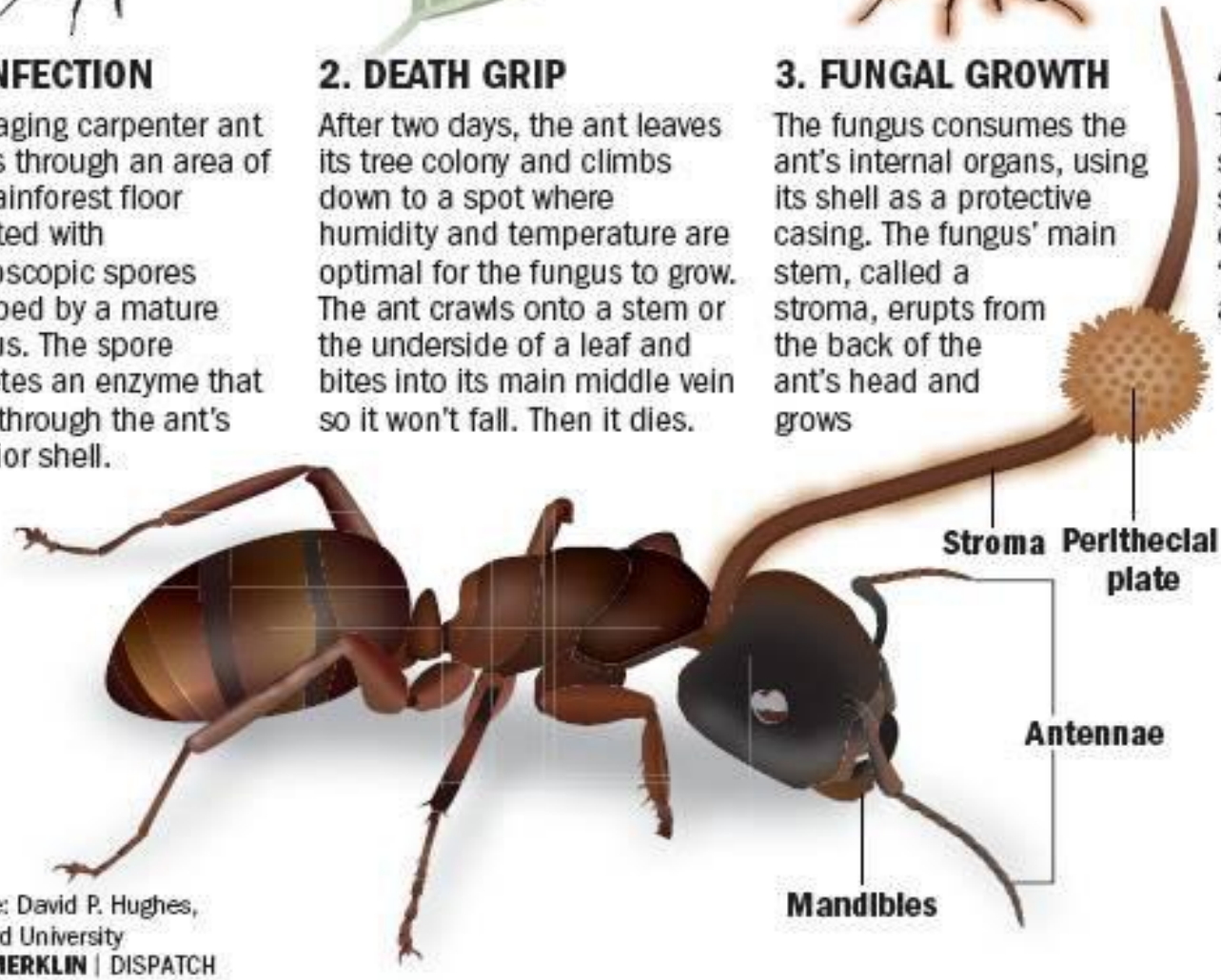
After two days, the ant leaves its tree colony and climbs down to a spot where humidity and temperature are optimal for the fungus to grow. The ant crawls onto a stem or the underside of a leaf and bites into its main middle vein so it won't fall. Then it dies.

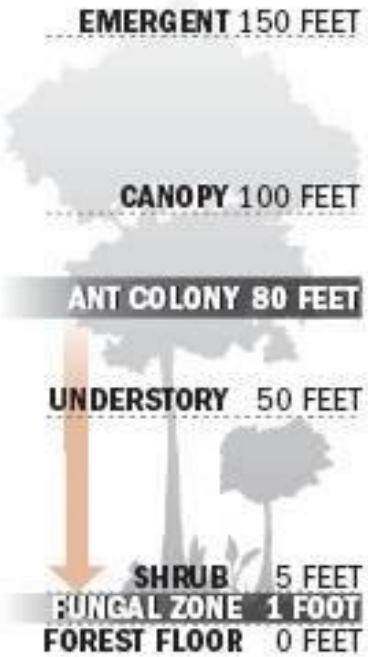
3. FUNGAL GROWTH

The fungus consumes the ant's internal organs, using its shell as a protective casing. The fungus' main stem, called a stroma, erupts from the back of the ant's head and grows

4. "KILLING ZONE"

The mature fungus releases spores from its stroma. The spores fall to the ground, creating a 10-square-feet "killing zone," which will attack new ants.



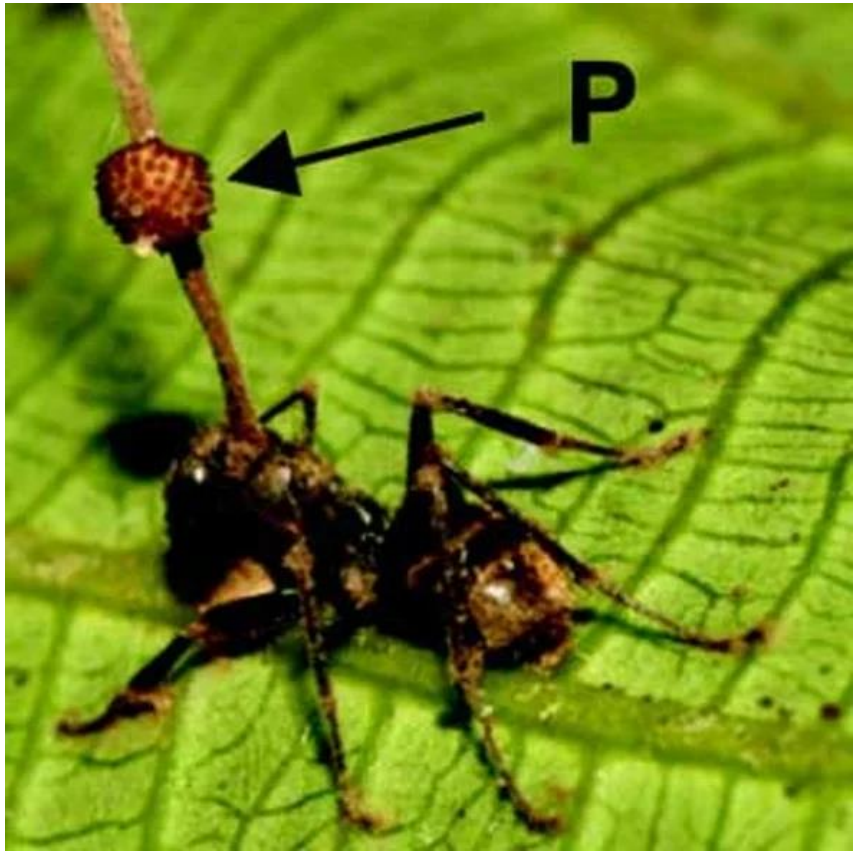


Source: David P. Hughes, Harvard University

LISA MERKLIN | DISPATCH

Photo by D. Hughes at Microbe Wikipedia. Used under creative commons licensing 2.5.

When the infected ant is positioned on a leaf at the location optimal for spore release, the mandibles clamp down permanently on a vessel, and they leave a characteristic scar. Hughes, Wappler & Labandeira (2010) reported the discovery in the Messel fossil site in Germany of 48-million-year-old leaf fossils showing several scars left by the mandibles of ants performing the death-bite directed by *Ophiocordyceps unilateralis.*



Entomophthora muscae

As the word “muscae” implies, this fungus is known for killing flies. Its strategy is similar in some ways to that of *O. unilateralis*. A sticky spore adheres to the fly, bores in, and causes the infected fly to position itself in a location favorable for the dispersal of spores.

The “fly” crawls up a plant, extends its mouth parts, and emits a kind of glue that attaches it permanently to the plant. Proliferating fungus distends the fly, and spores burst out from the weaker parts of the exoskeleton.

House flies are a common host, and you may find some glued to a window or windowsill now and then.

E. muscae was first identified 160 years ago, when a scientist found a lot of flies glued to his drapes.

The fungus positions the fly with wings spread high and wide, so as not to get in the way of spore dispersal.

When the dead fly is “ripe,” the fungus emits a chemical pheromone that draws healthy males to attempt to mate with the corpse, and thus get covered in spores.

E. muscae infects SW*D*

Massospora cicadina

M. cicadina is a fungus with great host specificity. Its host is the periodical cicada seen in parts of the US southeast and midwest.

Adult females lay eggs in little slits in the bark of small twigs. The eggs hatch in about 6 weeks, and the nymphs fall to the ground and burrow into the soil.

The nymphs of the cicada exist underground for years, sucking phloem from the roots of deciduous trees.

After the 17 or 13 year growth under ground, nymphs crawl up the tree trunk and undergo a final molt and emerge as adults.

M. cicadina is present in the soil under trees, so when the adults emerge to mate and lay eggs, some are already infected.

After mating, eggs are laid in bark slits, hatched new nymphs fall to the ground, burrow in, and so on.

Cicadas infected with *Massospora cicadina* are not bonded to a surface as in the previous examples, but their behavior is definitely altered to suit the fungus, rendering the cicadas “hypersexual.”

Normally male cicadas sing a “song”to attract mates and females flick their wings in a sexy way, but infected males sing continuously *and* flick their wings.

Fungal spore formation occurs in the distal abdomen, including the genitals. Thus mating spreads the infection.

The abdomen is so taken over by spore production that bits begin to fall off, including the genitals. Nevertheless the cicada continues to walk, fly and sing, shedding *Massospora* spores everywhere. One scientist called infected cicadas “flying saltshakers of death.”

https://inf.news/en/science/40e4a42de9be321fd4c3d9bfd2f6fd54.html

Lecanicillium lecanii

This fungus was until recently known as *Verticillium lecanii.* It is commercially available, and is primarily marketed for control of greenhouse aphids, whiteflies, and thrips. Products are available in other countries for control of lepidopteran, homopteran, and dipteran pests of flowers, vegetables, and other crops.

Its various strains infect a wide array of plant pests, representing the orders Orthoptera, Hemiptera, Lepidoptera, Thysanoptera and Coleoptera. They also infect some non-arthropods, such as nematodes and reptiles.

Lab studies showed one strain (ICAL6) gave 95% mortality in exposed *Myzus persicae.*

L. lecanii infects aphids, white flies , spider mites, other mites, and scale insects, and has a worldwide distribution. It is often used for pest control in greenhouses.

When a spore adheres to a prey insect, a hypha penetrates into the body of the insect and begins growing, secreting toxins and digesting. In a day or two the insect sickens and stops feeding and moving. Killing takes 3 to 5 days. Spores are formed among the hyphae progressively covering the insect's cuticle, and are transferrable, in the sense that spores from a fungus grown in an aphid can readily infect a spider mite, etc

https://academic.oup.com/annweh/article-abstract/30/2/209/321025?

Katyayani

- Katyayani Verticillium lecanii comes with higher CFU $2X10^8$ Thus More Powerful Liquid Solution.
- Katyayani Verticillium lecanii is Cost Effective because of high CFU Therefore low dose is required
- Katyayani Verticillium lecanii comes in Liquid Form thus Have High Shelf Life Comparatively.
- Katyayani Verticillium lecanii Liquid is Easy to Use and Dissolve quickly in water.

Other Verticillium lecanii

- Other product come with regular CFU $1x10^8$ CFU thus average result.
- Low CFU therefore high dose is required comparatively.
- Bio Fungicide Powder forms have low shelf life and typical conditions are required for storage.
- Other Forms may take time.

The high virulence and epizootic efficiency of some *Lecanicillium lecanii* strains towards certain insects has been used to develop several commercial mycoinsecticides.

Different strains of *L. lecanii* are commercially available under several trade names.

L. lecanii has also been the subject of ongoing genetic manipulation for the purpose of increasing its killing efficiency and altering its host range.

Metarhizium robertsii

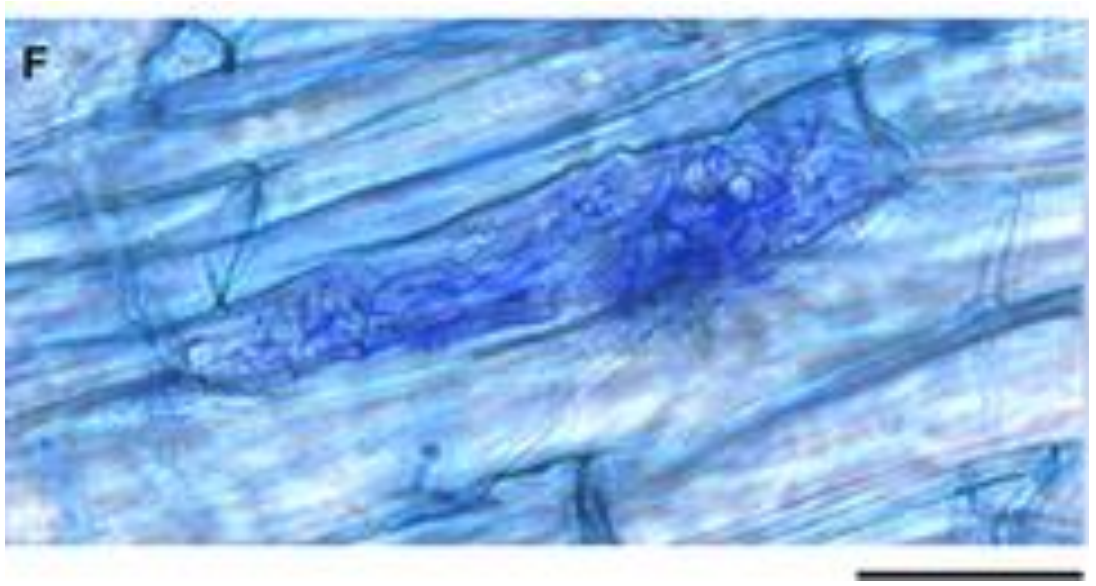
This fungus was previously known as *M. anisopliae.* It is found in soils worldwide. It was first identified in an infected beetle. The disease it causes is called green muscardine, due to the green coating of spores.

The first attempt to control a pest with a fungal agent was carried out in Russia in 1888, when the fungus now known as *Metarhizium robertsii* was mass produced on beer mash and sprayed in the field for control of the beet weevil.

In fact, *M. robertsii* has been observed to infect over 200 insect pest species. *M. robertsii* and its related species are used as mycoinsecticides to control a number of pests such as termites, thrips, etc, and its use in the control of malaria-transmitting mosquitos is being studied. *M. robertsii* does not appear to infect humans but has been reported as a significant pathogen of reptiles.

Metarhizium robertsii is registered in the U.S. for control of household cockroaches.

M. robertsii conidia can adhere to, germinate on, and colonize roots of some plants. Furthermore, plant roots treated with *Metarhizium* grew faster and the density of plant root hairs increased when compared with control plants. *Metarhizium* can be seen as a mycelial aggregate within root cortical cells as well as in the intercellular spaces, with no apparent damage to the plant.



M. robertsii Is thus an “endophyte,” living within plant cells. It lives in the rhizosphere as well, apparently attracted by root exudates, and can infect and kill insects in that area.

The final touch is that *M. robertsii* can act as a conduit to provide insect-derived nitrogen to plant hosts.

Thus *M. robertsii* has solved the chronic problem of predators: how to survive once you have killed all the prey. *M. robertsii* is fed photosynthate by the plant symbiont.

Behie, Zelisko & Bidochka (2012) showed the ability of *M. robertsii* to feed nitrogen to plants by placing waxmoths that had been raised on heavy nitrogen and infected with *M. robertsii* into the soil around bean or switch grass plants. The moths were separated from the plant roots by a mesh screen too fine for roots to pass through. After two weeks, N^{15} made up 28% of the nitrogen in the bean and 32% of that in the switchgrass. All of that nitrogen had previously been part of the waxmoths' bodies.

Butt, et al (1998) showed that honey bees could be dusted with dry *M. robertsii* spores using an automatic dispenser at the hive entrance, and then convey the spores to canola flowers, where the spores infected and killed the pollen beetles that feed on and lay eggs in the flowers. Killing of the beetles was very efficient under some circumstances. There was no evidence for any negative effect on the bees.

Beauveria bassiana

B. bassiana is another very widespread soil fungus, and its various strains encompass a very broad host range. It is available commercially as a mycoinsecticide. It causes a disease in insects known as white muscardine.

Once the spores invade the insect body, they multiply throughout, producing toxins and draining the insect of nutrients, eventually killing it. Once the fungus has killed its host, it grows back out through softer areas of the cuticle while covering the insect with white mold. This mold creates millions of new infective spores.

As a species, *B. bassiana* parasitizes a very wide range of arthropod hosts. However, different strains vary in their host ranges, some having rather narrow ranges, like strain Bba 5653 that is very virulent to the larvae of the diamondback moth and kills only few other types of caterpillars. Strains that do have a wide host range should be considered nonselective biological insecticides. These should not be applied to flowers visited by pollinating insects.

The first commercial formulations of *B. bassiana* were developed in 1995 using the strains GHA and ATCC 74040, which are sold under the trade names BotaniGard, Mycotrol, and Naturalis. Another strain, ANT-03, was isolated in 2000 and marketed in 2013 under the name BioCeres.

Each of the bottles pictured costs over $100 on Amazon.

The earliest appreciation of this entomopathogen was by Agostino Bassi (1773–1856) who noticed that some silkworm moth larvae suffered from a fungal disease, which he called white muscardine. The species *B. bassiana* was named after Bassi. The first *B. bassiana*-based insecticide for use against potato beetle and codling moth was produced in the USSR in 1965.

Beauveria bassiana is registered in the US to control grasshoppers, locusts, and Mormon crickets on rangeland, pastures, alfalfa, corn, cotton, potatoes, sugar beets, canola, and many others.

As of 2020, *B. bassiana* has the largest share of the mycoinsecticide market. *Metarhizium robertsii* has the second largest share. Their success can be attributed to their broad host ranges, and perhaps to their long history of use. However, different strains vary in their host ranges, some having narrow ranges.

For a full list of what mycoinsecticides exist, where they are registered and for what purposes, see Faria & Wraight (2007), in the reference list at the end.

Fungus	Grows in vitro	Meets a need?	Prey spectrum	Harms pollinators?	Harms humans?	OMRI listed?
Ophiocordyceps unilateralis	Yes*	No	Narrow	No	No	No
Entomopthora muscae	Yes, but…	Yes	Narrow*	No*	No	No
Massospora cicadina	Yes*	No	Narrow	No	No	No
Lecanicillium lecanii	Yes	Yes	Broad	No	No	Yes
Metarhizium robertsii	Yes	Yes	Broad*	No	No	Yes*
Beauveria bassiana	Yes	Yes	Broad*	No	No	Yes

Can Target Insects Evolve Resistance?

The development of resistance by insects is the plague of chemical insecticides. What about resistance to fungi?

For example, the ants targeted by *Ophiocordyceps unilateralis* are showing increasing grooming behavior to detach spores, and colony members now carry infected members far from the nest area.

House flies infected with *Entomophthora muscae* have now been observed to find a relatively hot spot to sit in for a while, and thus have sometimes cured their infections.

Unlike chemical insecticides, though, fungi are able to evolve as well, and can perhaps evolve spores that don’t attract attention, or heat tolerant variants.

What about the bees?

Ophiocordyceps unilateralis does not infect Hymenoptera, but its relative, *O. humbertii* infects social wasps and affects their behavior.

Entomophthora muscae does not infect Hymenoptera, nor do its genus mates.

Massospora cicadina does not infect Hymenoptera, nor do its relatives.

Lecanicillium lecanii apparently does not infect Hymenoptera, but little data seem to exist

Metarhizium robertsii evidently does not infect honey bees or other hymenoptera

Beauveria bassiana does not infect honey bees or negatively affect parasitoid wasps

Effects on *Varroa destructor*?

Ophiocordyceps unilateralis, *Massospora cicadina*, and *Entomophthora muscae* do not infect the varroa mite.

Lecanicillium lecanii did infect the mites when tested, but did not survive inside bee hives (too hot and dry).

Metarhizium robertsii had the same problem, but WSU scientists did selection for increasing heat tolerance ("directed evolution"), and have developed a strain that is lethal to *V. destructor* and can live inside the beehive (Han, et al., 2021).

Beauveria bassiana spores were found in mites in capped brood cells in 4 hives in Denmark. The spores recovered from the mites were viable and able to be cultured, which suggests that *B. bassiana* can survive in the hive. It is unknown whether the viability of the infected mites was affected. Outside the hive, varroa mites are susceptible to *B. bassania.*

Effects on Humans?

Through assessing the risks of infections, allergies, and poisoning/toxic effects, the most used mycoinsecticides such as *B. bassiana* and *M. robertsii* have been verified to be safe biocontrol agents.

But…

Inhaled fungal spores can cause asthma in people, and it is important to wear a mask while spraying or dusting, and to change clothes after applying a mycoinsecticide.

Entomopathogenic fungi produce “mycotoxins” in their infected hosts. It is unlikely a person would be affected by these, since they would be contained within the dead insects.

Most commercial mycoinsecticides specify a waiting period of about 4 to 12 hours before re-entering the sprayed area, but a wait of zero days before harvesting and consuming treated plant material.

Pros and Cons

Pests take a week (more or less) to die once infected. This gives the fungus time to get as much nutrition as possible from the prey

Products are expensive

Weather conditions can negatively impact fungal survival, especially sunshine.

Not all pests have treatments yet

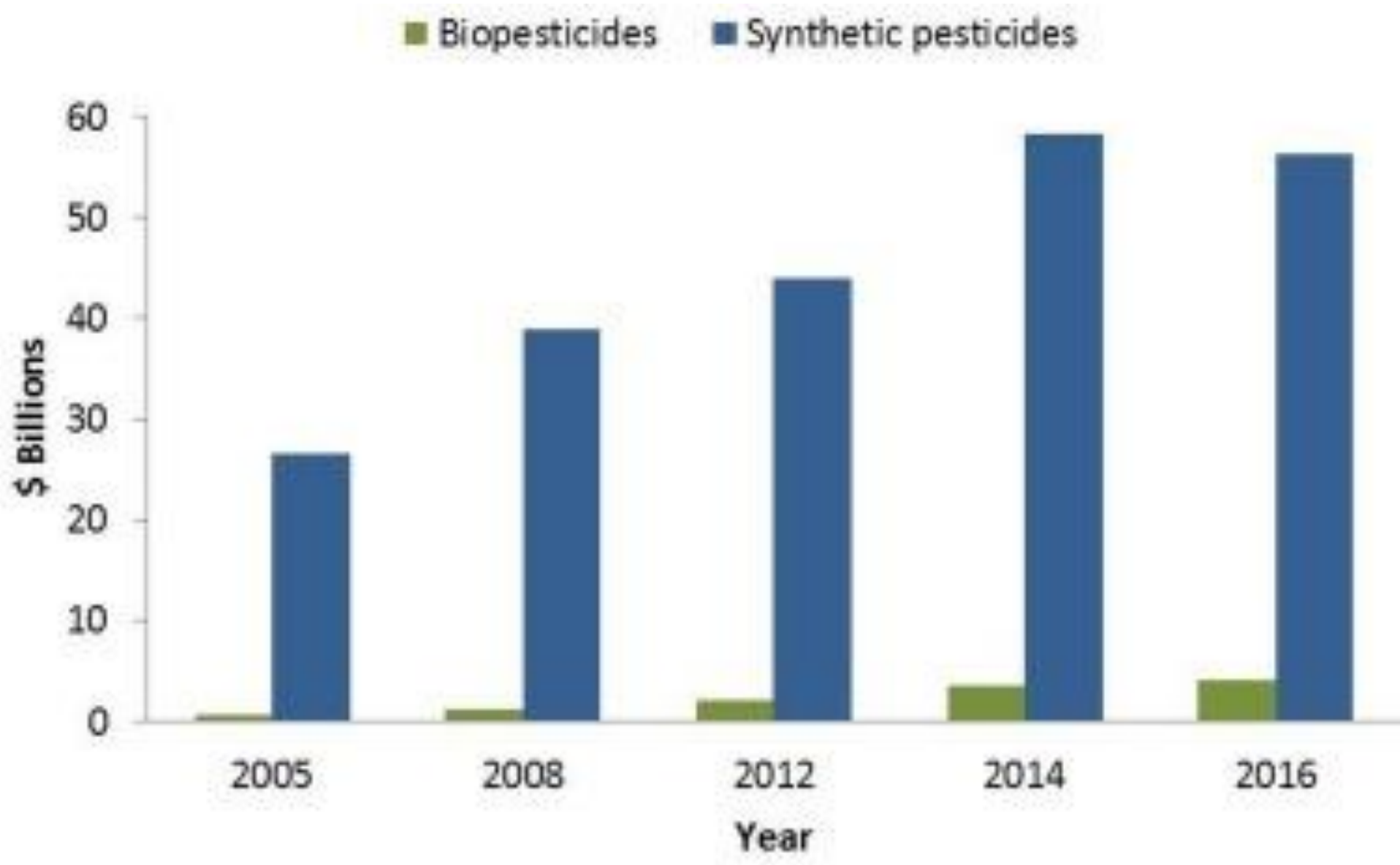
Can’t use fungicides

No toxic residuals, other than surviving infectious fungi.

Little collateral insect damage

No consequential damage to humans, pets, or beneficials

The fungal spores can be carried to untreated insects and increase the effective range of the treatment.


Biopesticides
Synthetic pesticides
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The Future?

Genetic engineering has entered the mycoinsecticide arena.
Fungal tolerance to UV radiation has been greatly improved by introducing into entomopathogenic fungi a gene complex from an archaean, which improves repair of UV induced damage to DNA.

Big gains in virulence have been obtained using genes encoding neurotoxic peptides. Putting a scorpion venom gene into a *Metarhizium robertsii* strain, for example, reduced time to kill by up to 40% and reduced lethal dosages of spores by 9- to 22-fold. Quicker killing was associated with smaller releases of spores, though. A *Metarhizium* strain expressing a toxin gene from a spider has been successfully tested against mosquito vectors of malaria in Burkina Faso.

Metarhizium acridum secretes a trehalase that uses trehalose, the main sugar in most insect hemolymphs. Overexpression of the trehalase gene accelerated the growth of *M. acridum* in the hemocoel of locusts, reducing time to kill by more than 8-fold compared with the wild-type strain.

Transfer of an esterase gene (*Metarhizium* esterase 1 or Mest1) from the generalist *M. robertsii* to the locust specialist *M. acridum* enabled the latter to expand its host range to include caterpillars.

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