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Can the Host's Immune Response Be Beneficial to the Parasite?

Microbiota–Parasite Interactions in Insect Pests



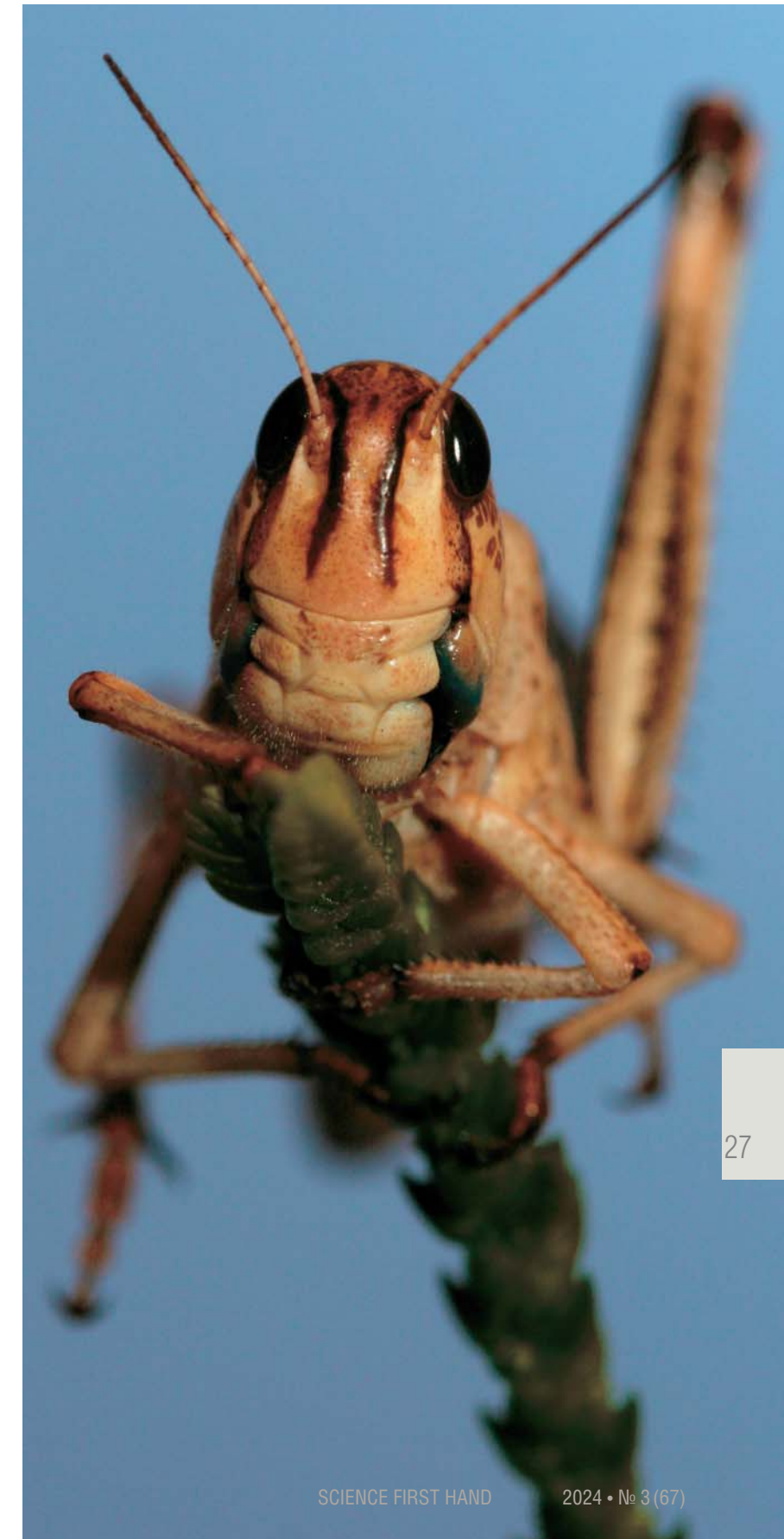
The history of pest control appears to have begun with the advent of agriculture, which dates back at least a dozen of thousands of years. Written sources tell us about ancient Sumerians and Egyptians fighting insects and ticks with sulfur. The ancient Greek poet Homer mentioned the use of fire against locusts, and the medieval philosopher and physician Avicenna described the insecticidal action of myrtle and wormwood.

Since the mid-19th century, the study and control of pests that destroy agricultural and ornamental crops, including insects, has built upon scientific knowledge. Substances of natural origin were gradually replaced by chemical insecticides, which helped solve the food problem for the rapidly growing humanity. But the “saviors” came with many disadvantages, primarily toxicity and indiscriminate destruction. Nevertheless, an alternative to chemical plant protection does exist, and that is bioinsecticides, i. e., bioactive compounds synthesized by living organisms or living organisms as such. These environmentally friendly pest control methods were practiced as early as 3000 or more years ago in China, where people used (and are still using!) predatory ants to protect their gardens from insect pests. Vigorous practical efforts and scientific research have been underway in this area since the end of the 19th century. So today's pest control engages a wide variety of living organisms: bacteria, fungi, animals, plants, etc. These organisms also include a good many parasites, which can enter into complicated relationships with their host insects, including its microscopic cohabitants, which cannot but influence the effectiveness of this “biological weapon”

Left: entomopathogenic fungus *Cordyceps militaris*. Photo by V. Glupov

Sphinx butterfly caterpillar with pupating parasitoid wasp larvae.
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Right: adult migratory locust (*Locusta migratoria*).
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Key words: insecticides, insect pests, immunity, microbiota, parasites, parasitoids, entomopathogenic fungi, Colorado potato beetle, waxy moth

Top right: Spraying a forest with an insecticide based on the bacterium *Bacillus thuringiensis* as part of a project to control *Choristoneura occidentalis*. 1988. Mount Hood National Forest, USA. Public Domain / W. Ciesla, USDA Forest Service, Region 6, State and Private Forestry, Forest Health Protection



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Insect pests can destroy up to 100% of agricultural crops, causing enormous economic losses; no less damage comes from forest pests. For example, in Siberia alone in 2017, the *Siberian silk moth* (*Dendrolimus superans*), a large-sized plain-looking butterfly, whose larvae feed on pine needles, damaged about 1.3 million hectares of forests.

These pests are usually controlled with synthetic insecticides, whereby the main problem is that insects may quickly develop resistance to these toxic compounds. Moreover, these methods lead to environmental pollution and could harm not only beneficial insects but also other animals and humans. One of the long-term adverse consequences of insecticides for human health is an increased risk of developing cancer as well as reproductive, immune, and nervous systems pathologies.

All this compels scientists to look for environmentally friendly methods to control insect pests. One such method is the use of specialized parasites hosted solely by insects and thus posing no threat to other animals, including warm-blooded ones.

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During a mass outbreak, gypsy moth (*Lymantria dispar*) larvae occupy vast territories and damage various types of trees, including fruit and coniferous ones.
Photo by V. Glupov





Migratory locust nymph that died from infection with the parasitic fungus *Metarhizium robertsii*. On the left is a colony of the *Metarhizium* fungus growing on an artificial nutrient medium. Photo by V. Kryukov

Insects are parasitized by the same groups of organisms (viruses, bacteria, fungi, protists, parasitic worms, or other arthropods) as mammals, but their diversity is much higher. The reasons are the large number of insect species, the lack of constant body temperature in insects, and a wide range of habitats.

Like in other organisms, the development of parasitic diseases in insects follows a complex multistage pattern depending on the characteristics of the parasite itself, on the physiological state and immune status of the host, and on environmental factors. Yet there is also an important difference; namely, infection in insects often leads to the host's death. The parasite often needs to kill its host to complete the life cycle and produce a new generation. This behavior is observed in some bacteria, in almost all parasitic ascomycete fungi (e.g., the well-known fungi of the

The migratory locust (*Locusta migratoria*) is a large (3.5–6.5 cm long) phytophagous insect. During the gregarious phase, this malicious grain pest can cover, within a day, tens of kilometers on the ground for larvae and hundreds of kilometers by air for adults. Today the migratory locust is controlled by a wide variety of methods: from chemical pesticides to biological products, such as Green Muscle against locusts, which is based on entomoparasitic fungi of the genus *Metarhizium*.

This genus of fungi comprises more than 80 species, which can invade insect pests from different taxonomic groups, including the Colorado potato beetle, wireworms, mole crickets, scarab beetles, etc. Among these fungi, there are both generalist species and those invading only certain taxa. Fungal spores are found in the soil of natural ecosystems and agrolandscapes; once they get on insect integuments, they germinate inside the body, invading the internal tissues.

***Metarhizium* is one of the most thoroughly studied insect pathogens; this fungus is discussed in thousands of experimental research papers and hundreds of reviews. There are over a hundred biological products made from these fungi. For instance, a large international team of scientists, technologists, and businessmen from Europe, North America, and Africa had been working for 13 years to create Green Muscle. The work was carried out within the Lubilosa research program, aimed at providing a biological alternative to chemical control of locusts. As a result, the team managed to enter the market with product forms protecting fungal spores, launch mass production, and find innovative methods of spraying**



Cordyceps is a genus of fungi, whose members parasitize mainly on insect's larvae, pupae, and adults. *Cordyceps* penetrates into the host's hemocoel, where it forms a distinctive outgrowth, i.e., the so-called stroma or the fruiting body. Fungi of related taxa are highly valued in Chinese medicine; in recent years, they have been sold as dietary supplements in Europe and the United States. The photo shows a moth pupa (*Notodontidae*) killed by *C. militaris*. Novosibirsk region. Photo by V. Glupov



The Colorado potato beetle (*Leptinotarsa decemlineata*) is a striking example of a dangerous invasive species that was brought to new territories by people. In the 1870s, this North American lover of plants from the *Solanaceae* family, which includes potatoes, tomatoes, and eggplants, travelled across the Atlantic in cargo ships and came to Europe, from where it spread as far as the Far East by the end of the 20th century.

Only adults of this species overwinter. They burrow into the soil in autumn and come up to the surface with the onset of warm days, whereby this period of surfacing may be quite long. Moreover, a part of the population can stay dormant in the soil for several years.

The Colorado potato beetle has relatively few natural enemies, partly due to the high amounts of toxins in its body as a result of the *Solanaceae* diet. One of the beetle's putative enemies is the virus *Leptinotarsa iflavirus 1*, first described from a beetle population in the south of Western Siberia. This infection is associated with characteristic symptoms. Dead larvae assume a typical pose; i. e., their body, as well as limbs, gets fully straightened, with their legs spread out in different directions. Based on preliminary data from Novosibirsk specialists, up to 60% of Colorado potato beetle larvae can die spontaneously from this viral infection. The iflavirus genome was recently assembled (Antonets *et al.*, 2024), which opens up prospects for developing new methods of biological control of this hazardous pest

Ant that died from infection with an entomopathogenic fungus. Photo by V. Glupov

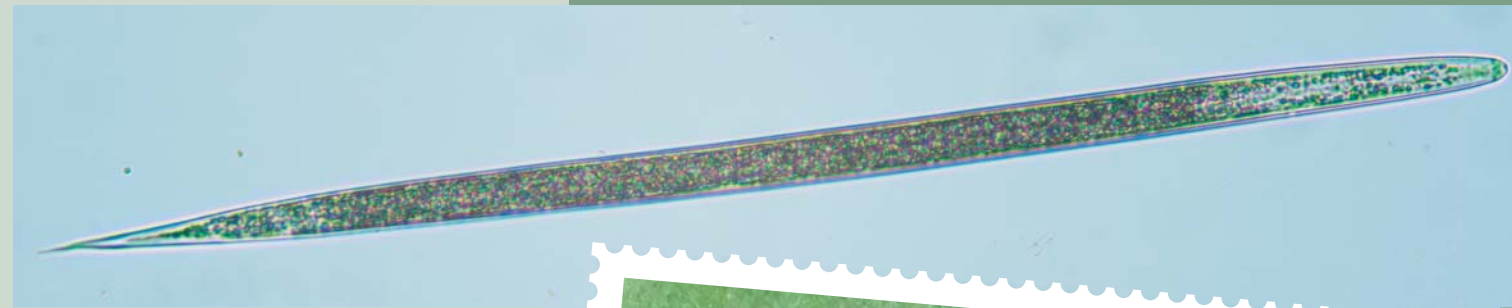
genus *Cordyceps*), and in many nematode roundworms.

A similar strategy is typical of parasitoids, i. e., organisms that spend a substantial part of their life on the cuticle or inside their host, feed on its tissues, and gradually kill it. Therefore, *entomophagous* parasitoids, hosted by insects, play an important role in integrated pest management. They are especially effective against cryptic plant dwelling insects, which use plants not only as food but also as a hide-out, which complicates pest control. These species include, e. g., the *cotton bollworm*, whose larvae "hide" in cotton bolls, or the corn borer, which overwinters in the stems of plants it has damaged.

Between microbiota and a parasite

The susceptibility of insect pests to parasites depends on a wide variety of factors. Firstly, it is the virulence of the pathogen itself, i. e., its ability to infect a given organism. Secondly, there are environmental factors, primarily UV radiation intensity, humidity, and temperature. Thus, on a sunny day, UV radiation can destroy pathogen spores in a few hours. The virulence of parasitic fungi and nematodes decreases at low humidity; their growth slows down at high and low (>35 and <10 °C) temperatures.

The effect of these factors matters most in outdoor pest management; greenhouses allow for more controlled conditions. In any case, these problems can be solved, e. g., by selecting strains resistant to high temperatures and low humidity and by introducing UV protectors into the formulations of biological products.



Entomopathogenic nematode roundworms penetrate the body of Colorado potato beetles overwintering in the soil. They infect the host with their symbiotic bacteria of the genus *Photorhabdus*, which suppress the host's immunity and secrete metabolites that make the beetles die. Dead individuals acquire a dull dark gray color; liquid flows out of them under pressure (right). The liquefied tissues of the beetle become a food source for the nematodes. In Western Siberia, these roundworms invade up to 10% of overwintering Colorado beetles. Above is a parasitic nematode isolated from a beetle overwintering in the south of the Novosibirsk region. Photo by P. Vlasenko and O. Yaroslavtseva



Such a pose of a Colorado potato beetle larva is associated with death from infection caused by the *Leptinotarsa iflavirus 1* virus. Photo by V. Kryukov



No less important is the health status of the insect pests themselves, primarily, their immunity characteristics and the composition and abundance of microbes inhabiting the insect body. It should be noted that recently, biology and medicine have been increasingly using such a concept as microbiota, which means the entire community of MICROorganisms inhabiting a MACROorganism. Another popular term – *microsymbiocenosis* – draws attention to the complex relationships both in-between the microbial associations themselves and between them and the host and the environment. It is these relationships that largely determine the ability of the host organism to ensure its homeostasis, i.e., the vitally important constancy of internal environment.

The microbiota of insects, like that of mammals and other vertebrates, participates in a whole range of important functions, including digestion, signaling, detoxification, protection from pathogens, etc. In insects, like in other animals, the most numerous and diverse microbial communities are those of the intestine and integuments, represented by a multilayered cuticle, which serves as an external skeleton. The internal organs of insects are often non-axenic either; i.e., they contain both intracellular and freely circulating microbes.

Importantly, the microbiota composition depends on nutrition as well as other conditions and can undergo substantial restructuring during the life cycle or when infected with certain pathogens. In the latter case, this community can play a dual role.

Bacteria: patriots and opportunists

There are known examples of insect symbiotic bacteria increasing the resistance of the host insects to parasitic fungi or parasitoids.

Thus, *Hamiltonella defensa*, a symbiotic bacterium of aphids, protects its host from its worst enemy, i.e., the parasitic wasp *Aphidius ervi*. Adult wasps lay their eggs in aphids' bodies, and the larvae that develop inside kill their hosts. The hamiltonella produces a toxic protein leading to the death of wasp larvae, with the toxin being encoded in the genome of a bacteriophage virus symbiotic with the bacterium rather than the bacterium itself (Oliver *et al.*, 2009).

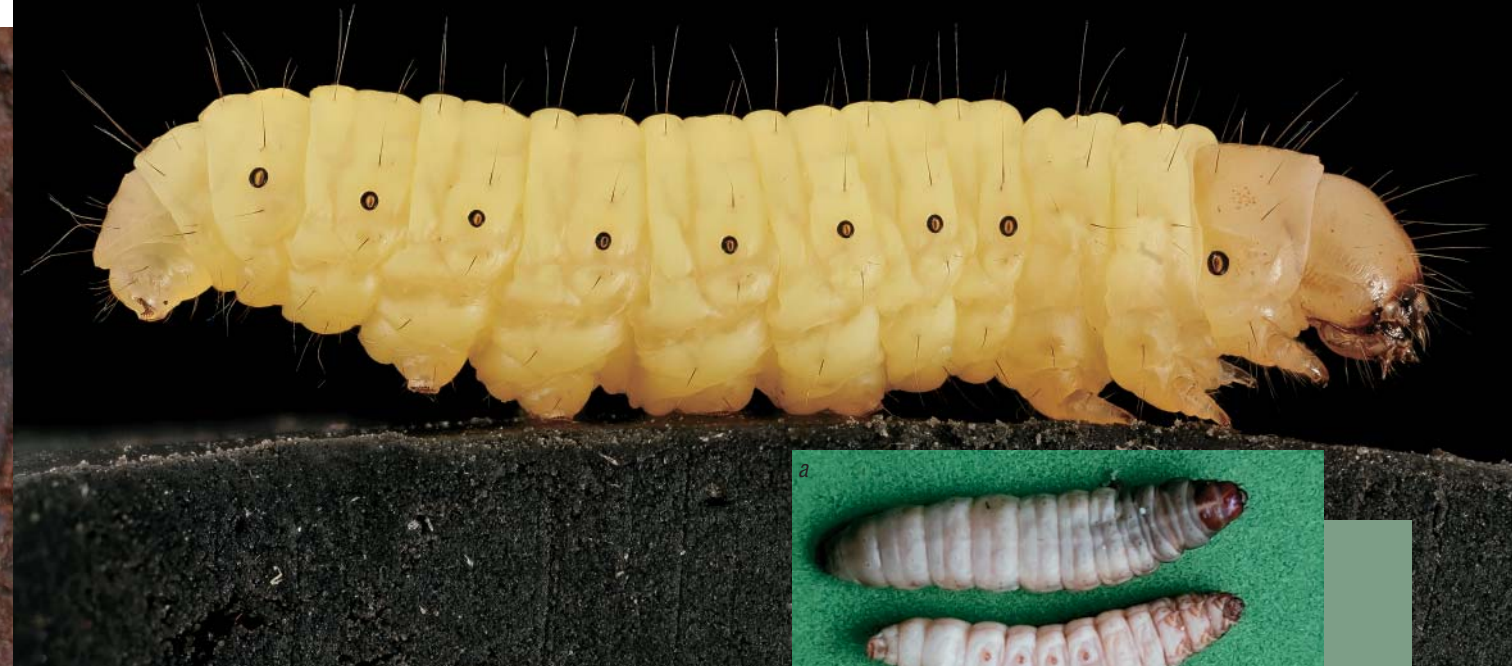
Other intracellular aphid symbiotic bacteria – *rickettsia* and *spiroplasma* – increase the resistance of these insects to fungi of the order *Entomophthorales*, most of which are parasites. In the case of a bad outcome, these bacteria at least prevent the proliferation of these fungi on the body of dead insects (Lukasik *et al.*, 2013).



Wax moth is a species in the family of pyralid moths. Above is an adult moth.
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The wax moth (*Galleria mellonella*) is considered to be a pest although it usually settles in abandoned hives. The larvae of this moth serve as a popular model object for studying infectious diseases. The larvae are active in a wide (19–42 °C) temperature range, but their optimum is 37 °C, which is close to human body temperature. Because of these properties, the wax moth can be used in modeling the development of not only parasitic diseases of insects but also various bacterial and fungal infections of humans, caused by pathogens such as salmonella, clostridia, *Candida* fungi, etc. These insects are relatively easy to breed en masse for laboratory manipulations

Yet another example is the antagonistic relationship between bacteria inhabiting the ant cuticle and parasitic ascomycete fungi infecting the insects through the integuments. In experiments, using antibiotics to remove bacterial biofilms from the ant cuticle increased the insects' susceptibility to these fungi (Masotto *et al.*, 2012).



Wax moth larvae can grow up to 1.8 cm in size. They live in the hives of honey bees, feeding on honey, bee bread, and wax combs. Public Domain

Photo:

- (a) wax moth larvae infected with the entomopathogenic fungus *Cordyceps militaris*;
- (b) larvae that died from infection caused by bacteria of the genus *Enterobacter* and *Enterococcus*;
- (c) a larva with a developed entomopathogenic fungus *Cordyceps farinosa* (spore-bearing outgrowths are visible on the surface of the larva's body).
Photo by V. Kryukov



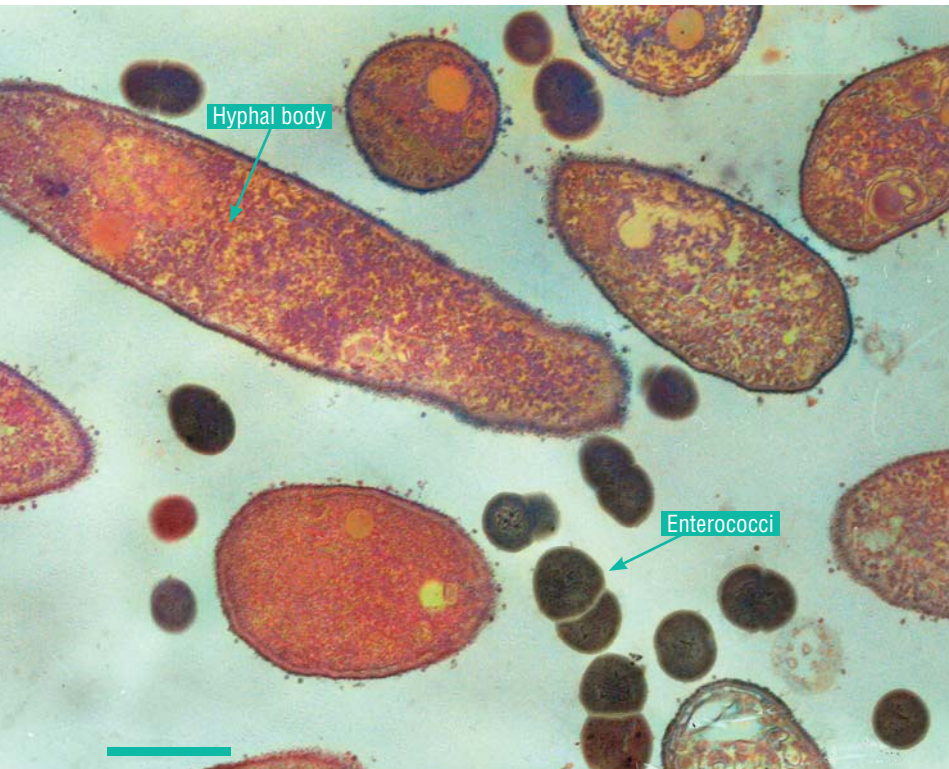


Image of the hemolymph of a wax moth larva with a combined infection caused by the fungus *Cordyceps militaris* and the bacterium *Enterococcus faecalis* shows fungal hyphal bodies and bacterial cells. Electron microscopy. Photo by A. Miller. Color retouching by the editors

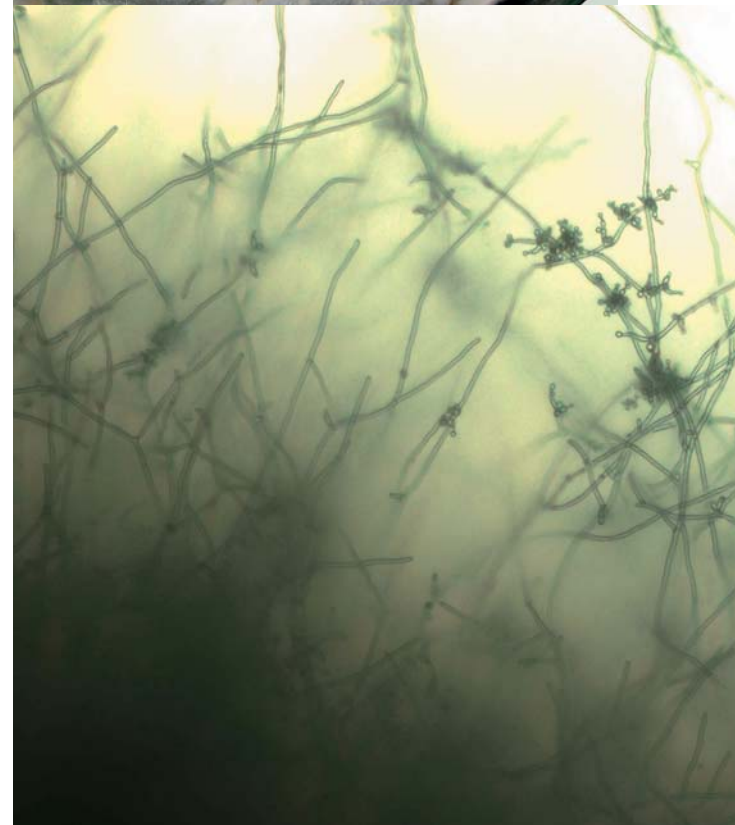
However, in some cases, events develop in a different scenario. A disease, including a parasitic one, leads to certain changes in the insect's body, namely, in the functioning of its immune and other systems, accompanied by destructive changes in the tissues, which may provoke vigorous proliferation of some symbiotic microbes. Once these microbes – many of which are normally harmless and are thus referred to as *opportunistic* (conditionally pathogenic) microbiota – find themselves in untypical organs and tissues, they themselves become pathogenic. And this complicates the development of parasitosis.

Thus, when widespread parasitic fungi of the genus *Beauveria* penetrate through the cuticle inside the hemocoel of malaria mosquitoes *Anopheles stephensi*, this invasion weakens the insects' intestinal immunity. The resulting decrease in the production of reactive oxygen species and antimicrobial peptides in the mosquitoes' intestines leads to dysbiosis, accompanied by a proliferation of enterobacteria *Serratia*. The bacteria penetrate from the intestines into the host's hemocoel, which accelerates the development of mycosis and leads to the quick death of the mosquitoes (Wei *et al.*, 2017).

A similar pattern of infection with this fungus is observed in the red turpentine beetle (*Dendroctonus valens*), a dangerous pest of conifers, which attacks weakened trees. Penetrating through the integuments, the fungus provokes accelerated proliferation of *Erwinia* bacteria in the intestines of the beetle's larvae. Experiments in feeding these bacteria to axenic larvae revealed the increased susceptibility of the latter to the parasitic fungus (Xu *et al.*, 2019). Similar phenomena were also noted in the gypsy moth–intestinal symbionts–*Beauveria* fungi system (Bay *et al.*, 2023).

In recent years, evidence has come to light that the parasite may be capable of controlling the microbiota of the host organism to prevent the development of opportunistic microbes. The parasite is interested in keeping the host alive as long as possible so that it can successfully complete its development. Since the rate of proliferation of bacteria forming the core of the microbiota is much higher than that of parasitic fungi or parasitoids, the latter must somehow prevent the growth of the bacteria and their penetration into the host's hemocoel. This can be achieved either by synthesizing metabolites that directly suppress bacterial growth or by manipulating the host's immunity (Fan *et al.*, 2017; Wang *et al.*, 2023).

A separate group of parasitic organisms are parasitoids, which use a special strategy. A parasitoid always kills its host eventually, albeit slowly, whereas a true parasite does not benefit from its host's death



“Live preserves” for parasitic wasps

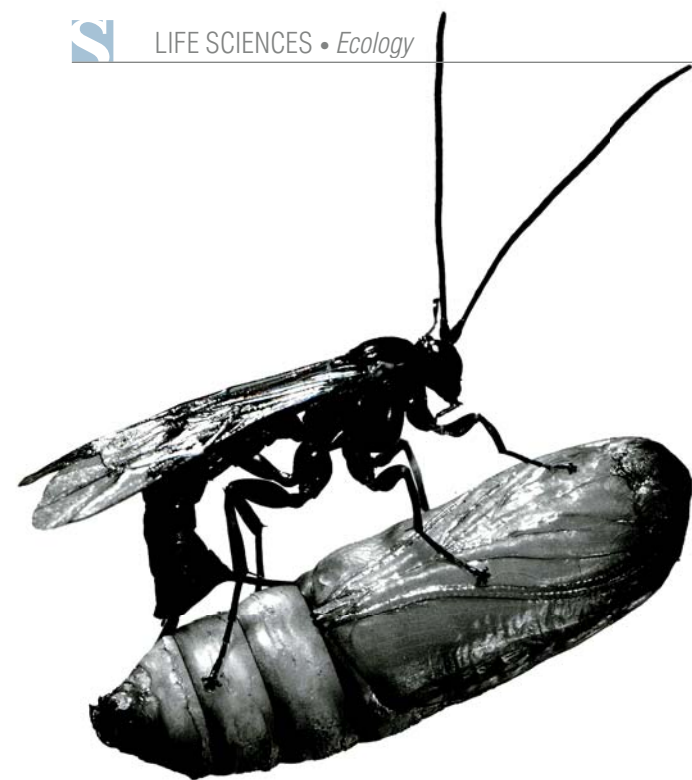
It was long believed that the immunity of an insect can only be manipulated by *endoparasitoids*, whose larvae live inside the host's body and directly contact with its immune system. As for *ectoparasitoids*, which develop on the integuments, they remain far less understood from this perspective.

The aforementioned parasitic wasps stand out in that their larvae can develop both inside and on the surface of their host. Meanwhile, the wasps can manipulate the host's immunity with metabolites produced either by adults or larvae themselves or by their symbiotic viruses.

Viruses that enter the host's body when the wasp lays eggs produce proteins that affect the host's immunity and

Strain Sar-31 of the fungus *Beauveria bassiana* (one of the most common entomopathogens) is used to obtain experimental biological products against phytophagous insects. Isolated in the south of the Novosibirsk region. Bottom left: fungal sporulation. Light microscopy. Photo by V. Kryukov



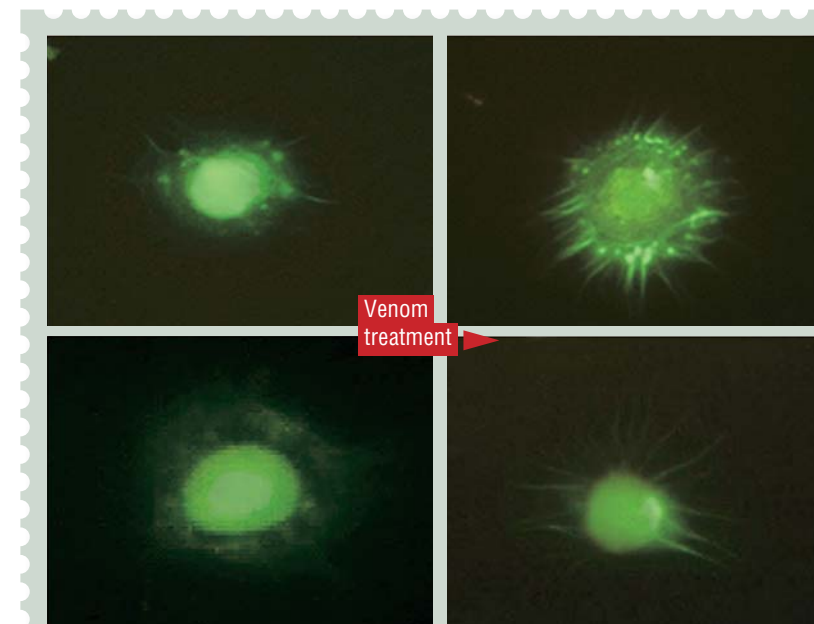


The *Braconidae* family of parasitic wasps includes about 21,000 species, including ecto- and endoparasites. Many species of this family parasitize on insect pests that damage forest trees and agriculture crops, which is why these wasps are often used in biological pest management. The miniature (2.5 mm) adult parasitoid wasp *Habrobracon hebetor* lives on average 20 days or less. The female, which feeds on nectar and insect hemolymph, can lay up to 18 eggs per day. The hosts for the parasitoid's larvae are *Lepidoptera* (mainly moths); the female is able to detect a potential host at a distance as large as half a kilometer. This entomophage is widely used to protect crops in open grounds and greenhouses; in grain storage facilities; for dried fruits, etc. It is believed that releasing 700–2000 adults of *H. hebetor* per 1 ha of crops is enough for effective control of pests

Female parasitoid wasp Apechthis ontario lays eggs in a wax moth pupa. 1962. Oregon, USA. Public Domain/ R. Ryan



Parasitic wasp of the *Braconidae* family. © CC BY 2.0/ Katja Schulz



Parasitoid *Habrobracon hebetor* paralyzes a wax moth (*Galleria mellonella*, top) larva with venom. The eggs laid by the female on the host hatch into larvae, which begin feeding on the hemolymph of the insect, which remains immobilized yet alive. Photo by V. Glupov

Treating wax moth hemocytes with the venom of the parasitic wasp *H. hebetor* changes the structure of the actin cellular cytoskeleton changes, which exhibits stress fibrils and nodules. Fluorescence microscopy. Photo by N. Kryukova

physiology, a phenomenon that largely eases the existence of the parasitoid larvae. This strategy is used primarily by endoparasitoids.

Ectoparasitoid wasps rely on another weapon, i.e., the venom produced and accumulated in the special glands of females of these parasitic hymenoptera. With this venom, they only paralyze their host without killing it – this is how the female prepares a kind of “live preserves” for its offspring.

The venom's components can change many of the host's physiological processes, as a result of which the host ceases to develop; its immune system becomes suppressed; and its metabolism synchronizes with the development of the parasitoid larvae. However, immune system suppression can create favorable conditions for a proliferation of opportunistic microbiota. In this case, the story will have a different end; i.e., the host will die prematurely, and the future generation of the parasite will die as well.



Habrobracon: a great manipulator

In order to assess the immune response of insects to ectoparasitoids, specialists from the Institute of Systematics and Ecology of Animals (Novosibirsk) conducted experiments on a model that included the parasitic wasp *Habrobracon hebetor*; its host, the wax moth (*Galleria mellonella*); the host's symbiotic microbiota; and various species of entomopathogenic fungi.

A female habrobracon first injects potential hosts (in this case, the moth larvae) with venom and then attaches onto them its eggs, from which the parasitoid larvae hatch. The latter feed on the surface of the moth larvae's surface, gnawing through the cuticle and sucking out their *hemolymph*, i.e., a substitute for blood and lymph, which circulates in the hemocoel of an insect. It is important that the hosts remain immobilized yet alive until the end of their days. The wasp, as a rule, prepares much more of these "preserves" than it needs for laying eggs.

Observations showed that the paralyzed wax moth larvae rarely die from spontaneous bacterial diseases although after envenomation they experience a powerful proliferation in their own microbiota: *enterobacteria* in the intestine and *enterococci* on the cuticle. However, signs of bacterial infection, indicating the entry of bacteria into the hemocoel, manifest themselves rarely (Polenogova *et al.*, 2019; Kryukov *et al.*, 2022).

The paralyzed moth larvae show a drastic (by the factor of 5,000–500,000!) increase in susceptibility to parasitic fungi of the genera *Beauveria* and *Metarhizium*. Meanwhile,

Body of a large (about 12 cm) caterpillar of the Indonesian cinquefoil butterfly with visible cocoons of a parasitic wasp of the genus *Apanteles* in the family *Braconidae*. © CC BY-SA 2.0/ gbohne

they turn out to be insusceptible to infection by less dangerous, opportunistic fungi (of the genera *Aspergillus*, *Fusarium*, *Penicillium*, etc.) (Kryukov *et al.*, 2013, 2017, 2022).

Why do the paralyzed larvae remain resistant to opportunistic pathogens but become very susceptible to more specialized ones?

The explanation lies with the good functioning of the immune system in their cuticle and intestines. This is evidenced, for instance, by the increased synthesis of antimicrobial peptides, i.e., compounds that disrupt the integrity of the cell wall in microorganisms (Polenogova *et al.*, 2019; Kosman *et al.*, 2024). These immune responses can effectively prevent bacteria and other opportunistic pathogens from penetrating into the host's hemocoel, so that the host does not die before the parasitoid completes its development.

As for more specialized pathogens – *Beauveria* and *Metarhizium* fungi – they are much better at overcoming the host's immune response. Moreover, the venom's influence



Destruction of the fat body

Slowing down of hemolymph circulation

Mass proliferation of bacteria in the gut and on the cuticle

Increased humoral immunity

Change in the composition of epicuticular lipids

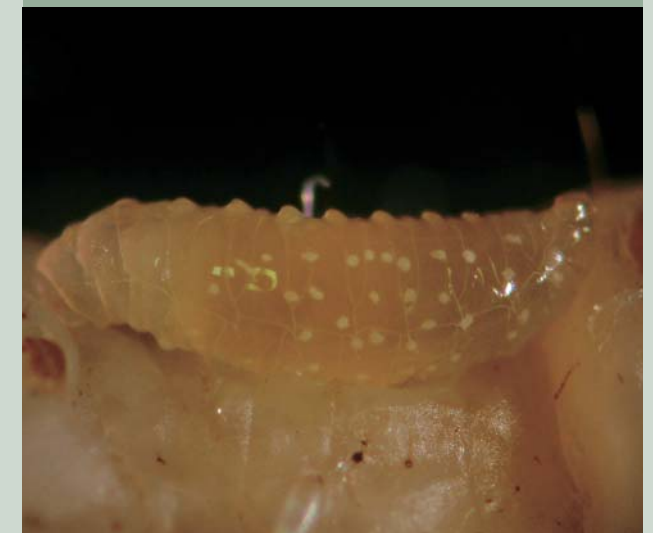
Decreased cellular immunity

Defense against bacterial infection

Protection from opportunistic pathogens

Successful development of parasitic fungi

Successful development of the parasitoid



When a wax moth larva is paralyzed with the venom of the parasitoid *Habrobracon hebetor*, its body undergoes several physiological changes, including the induction of certain immune responses. On the one hand, these changes prevent the proliferation of opportunistic bacteria and fungi, maintaining the viability of the paralyzed larva. On the other hand, they enable the parasitoid (and more specialized entomopathogenic fungi) to successfully complete its development cycle.

Colorado potato beetle: a feeder of parasitic fungi

leads to a change in the lipid composition of the cuticle and to a decrease in the rate of hemolymph circulation and the adhesive capacity of hemocytes (Kryukov *et al.*, 2018, 2022). All these effects make it easier for the parasitic fungi, which are capable of utilizing specific components of the insect cuticle, to penetrate into the moth larvae's hemocoel.

So far it is difficult to say what exactly stimulates the immune response of the paralyzed moth larvae: rapid proliferation of their own microbiota or the effect of the parasitoid's venom components. Regardless of the reason, we see that the immobilized host not only serves as a source of food for the wasp larvae but also actively responds to the proliferation of potentially dangerous microorganisms. It is obvious that in this case, the host's immunity works in favor of either the parasitoid or specialized fungi, giving them the opportunity to go through a full development cycle using the host's resources.

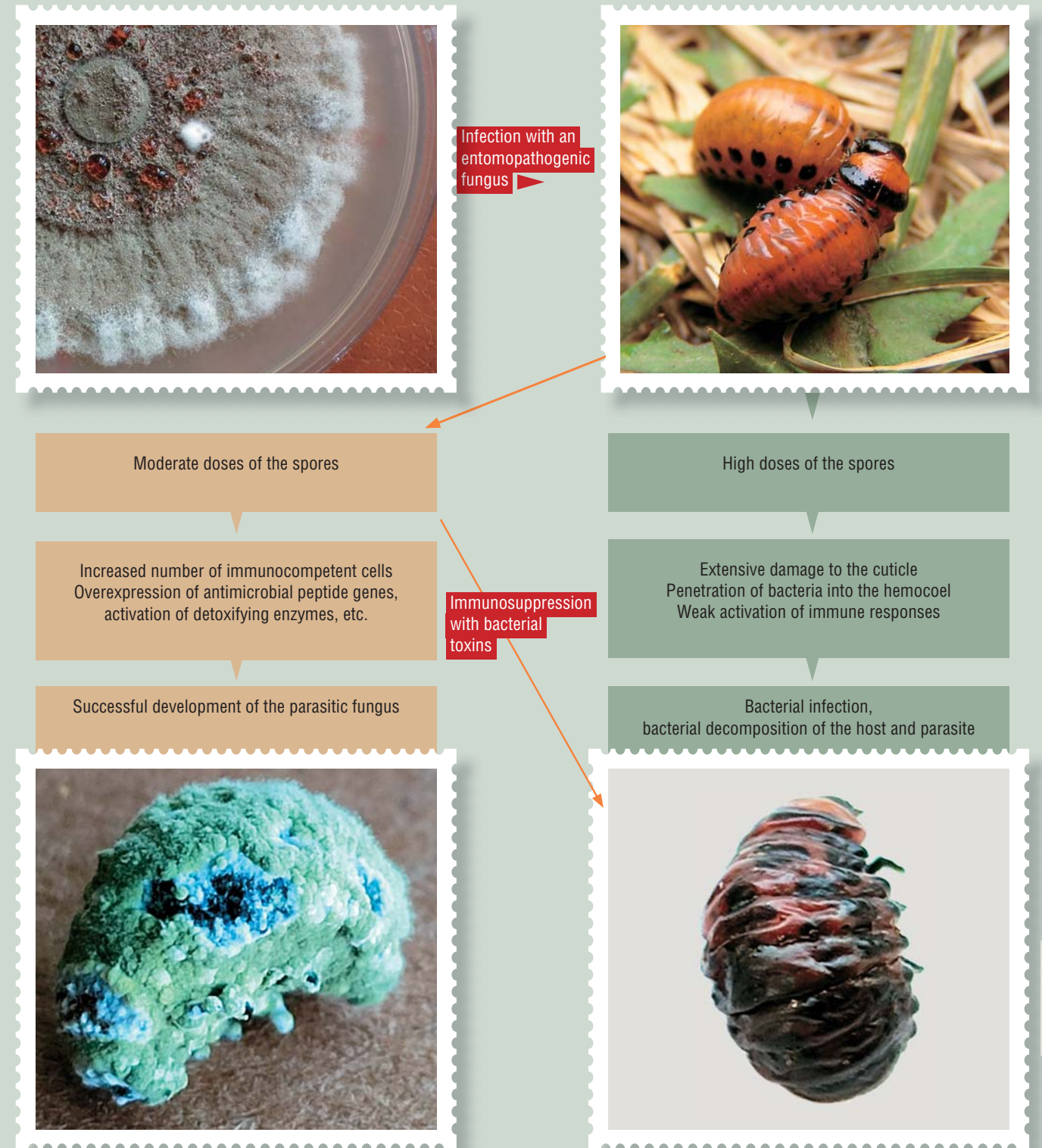
During the breeding season, a female Colorado potato beetle can lay up to 500–1000 eggs; in southern regions, this beetle can produce 2–3 generations per summer.
© CC BY-NC 2.0/ Brad Smith



Another series of experiments used a model that included larvae of the *Colorado potato beetle*, its bacterial cohabitants, and the entomoparasitic fungi *Beauveria* and *Metarhizium*.

Immune responses in the larvae were suppressed with *avermectins*, i.e., metabolites of the soil actinobacteria *Streptomyces avermitilis*, or with the endotoxins of the *Bacillus thuringiensis*. It is known that avermectins are widely used as insecticides. In addition to their neurotoxic effect, they suppress cellular immunity and cause digestive disorders, as well as general delay in insect development. As for the endotoxins of *B. thuringiensis*, they damage the intestinal epithelium, facilitating the penetration of bacteria into the hemocoel.

After the beetle larvae were infected in the experiment with moderate doses of parasitic fungal spores, mycosis developed according to the "classical" scenario: the larvae died starting from the sixth or seventh day after infection. Meanwhile, fungal hyphal bodies fully colonized the larvae's hemocoel, forming a "sclerotium," which then produced abundant sporulation on their integuments.



After infection, the following immune responses were induced, at certain stages, in the host's organism: a enhancement of the total number of hemocytes; increased synthesis of enzymes associated with the inactivation of toxic metabolites; enhanced expression of the genes related with immune-signaling pathways, specifically Toll (defence against fungi and gram-positive bacteria), IMD (mainly against gram-negative bacteria), and Jak–Stat (against viruses as well as fungi and bacteria).

However, when these immune responses were suppressed using the aforementioned immunosuppressants, the majority of insects died much earlier, with symptoms of bacterial decay rather than mycosis (Kryukov *et al.*, 2009; Tomilova *et al.*, 2016; Yaroslavtseva *et al.*, 2017).

One of the reasons for this outcome is as follows. When the fungi germinate through the cuticle, the local bacteria penetrate into the larvae's hemocoel (Vey and Fargues, 1977). Since bacteria proliferate faster than fungi, the latter lose if the host dies from bacterial infection. So the host's immune responses in the case of "classical" mycosis, which stop the bacterial attack, are absolutely necessary for the parasites to develop normally and complete their life cycle.

A similar pattern was also observed in this model with excessively high concentrations of entomopathogenic fungi spores. In this case, the beetle larvae also died with symptoms of bacterial infection within 3–5 days after infection. In addition, the larvae exhibited extensive deformations and damage to the cuticle, a severe weakening

of the organism, and a decrease in the activity of the genes with the IMD and Jak–Stat immune-signaling pathways. Against this background, a sharp increase was observed in the number of bacteria on the insect's integuments and in its hemolymph, indicating the penetration of bacteria into the hemocoel. In the absence of pronounced immune responses, bacteria gained an advantage, which led to the death of the host and the decomposition of its tissues. So, in this scenario too, parasitic fungi failed to complete their life cycle (Kryukov *et al.*, 2024).

These results agree well with the data obtained by other authors in recent years. For example, after infecting larvae of the cotton bollworm (*Helicoverpa armigera*) with the fungus *Metarhizium rileyi*, opportunistic bacteria were found to translocate from the intestines into the hemocoel, where they were competing intensely with the fungus for the host's food resources (Wang *et al.*, 2023). However, in the meantime, the larvae began to vigorously synthesize antibacterial peptides. The authors explain this fact with the change in hormonal regulation caused by the fungus and with the engagement of several general receptors

Parasitoid wasps, which have completed their development cycle in an insect pupa, are emerging from their dead host. © CC BY-SA 2.0 / karen johns



Female of the parasitoid wasp *Aphidius rhopalosiphii*. Members of the genus *Aphidius* are widely used in biological control programs against aphids. © CC BY-SA 2.0 / Gilles San Martin

of immune-signaling pathways in response to the invasion of the fungal and bacterial pathogens.

In general, the results obtained suggest that the "classical" mycosis scenario requires moderate infection doses in order to fully engage the host's immune responses. Only then will the parasite develop successfully in the insect pest. However, the application of parasitic fungi as bioinsecticides to quickly destroy pests requires the use of immunosuppressants. The latter can change the course of the pathological process towards a combined infection (*coinfection*), which causes rapid death.

Thus, experiments on model systems clearly show that in insects, interactions between their microbial communities and parasites can be both antagonistic (when symbiotic microbes enhance the host's resistance to the parasite) and synergistic (when these microbes participate in the pathogenesis).

In the latter case, these microorganisms end up in untypical tissues of the insect's body, thereby complicating the course of the parasitic infection or even changing its scenario. Sudden development of a bacterial infection can kill both the host and the parasite.

However, insects infected with parasitic fungi or parasitoids are often capable of an active immune response, which helps to avoid excessive bacterial proliferation and subsequent destruction of the host's organs and tissues. In this case, the host's immunity undoubtedly works in favor of the parasites, allowing them to successfully proliferate.

The different approaches to manipulating insect immunity – towards the development of either the main parasite or opportunistic infections – may find application in biological control of pest insects.

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