Biocontrol Microbes in Plant Protection

Sir: what is BT – I always thought it was British Telecom?

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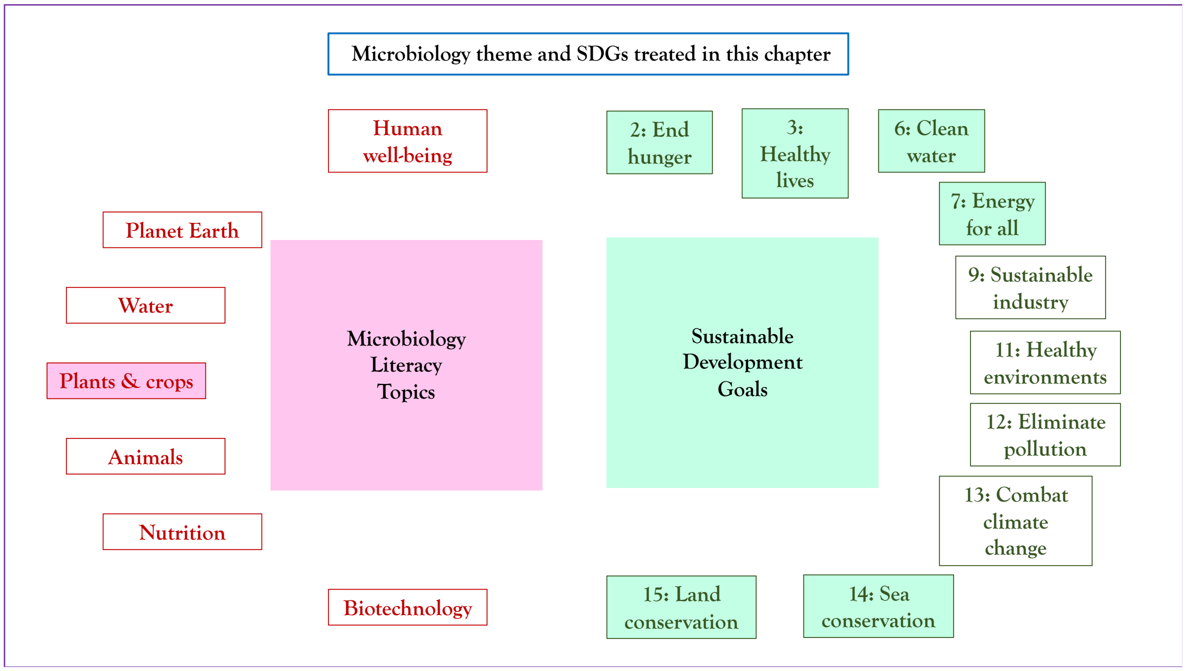
Storyline

Sustainable agriculture depends on crop protection from insect attack. Crop protection from insect pests has been a fundamental activity to increase yields and quality of human food. Chemical insecticides have been used extensively to eliminate or reduce insect infestations and assure a successful crop production. However, most chemical insecticides are toxic to many other organisms, such as beneficial insects, but also to humans and all kinds of animals and plants. In addition, they accumulate in the environment for quite long periods of time. Thus, strategies to reduce the use of chemical insecticides in plant protection are urgently needed. Microbial insect pathogens could be a good alternative for reducing the use of toxic chemicals in agriculture. Pathogens that have been shown to be specific for insects include viruses, fungi and bacteria. All these microbial pathogens of insects have been used for developing insecticidal products that may replace the chemical insecticides used for insect control in agriculture. The most effective and successful insect pathogen used for pest control is the bacterium *Bacillus thuringiensis* (Bt). The members that belong to Bt bacteria show a narrow insect specificity but highly efficient control properties. Moreover, genes that are responsible for the insecticidal activity of these insect pathogenic bacteria have been introduced into the genome of different crop plants, thereby endowing plants themselves with insect resistance. The use of microbial pathogens of insects, or the genes from these microbes, can improve agricultural production and also constitute a means of reducing the use of toxic chemical insecticides in agriculture, thus providing an important contribution to Sustainable Developmental Goals.

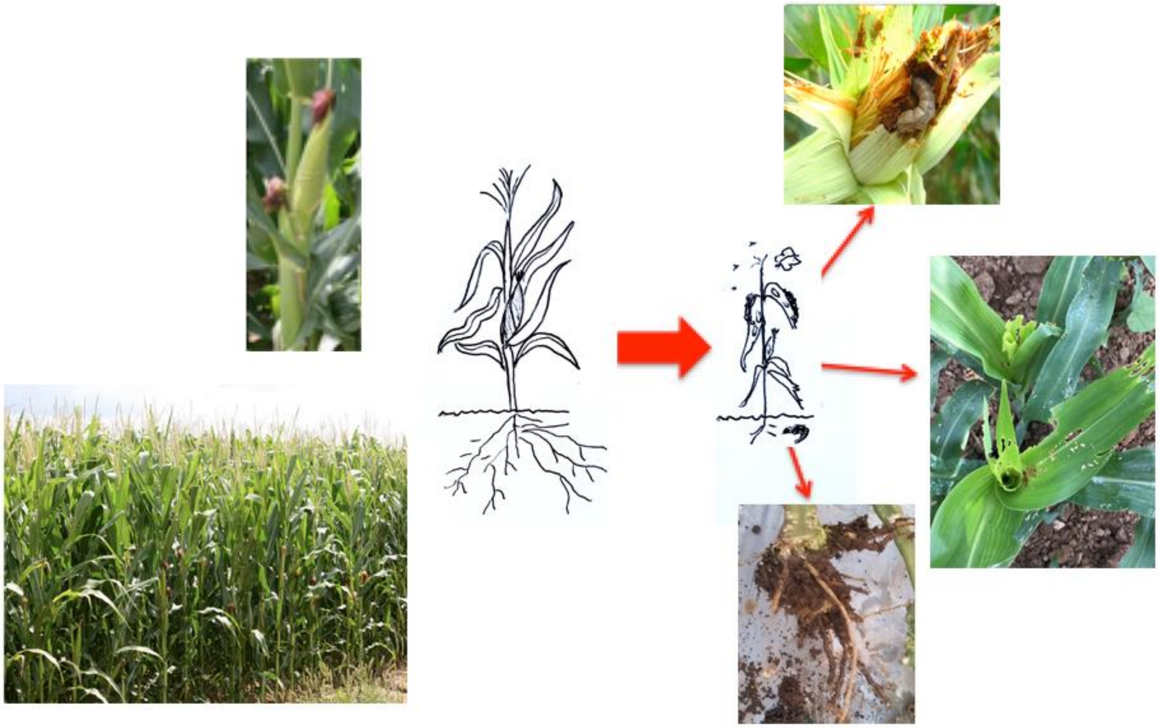


The Microbiology and Societal Context

The microbiology: Microbial insect pathogens as powerful insecticides; microbial insect pathogens as source of genes for crop protection in genetically modified plants. *Peripherally for completeness of the storyline*: the business of bioinsecticidal products, organic agricultural practices. *Sustainability issues*: food, health; energy; environmental pollution.

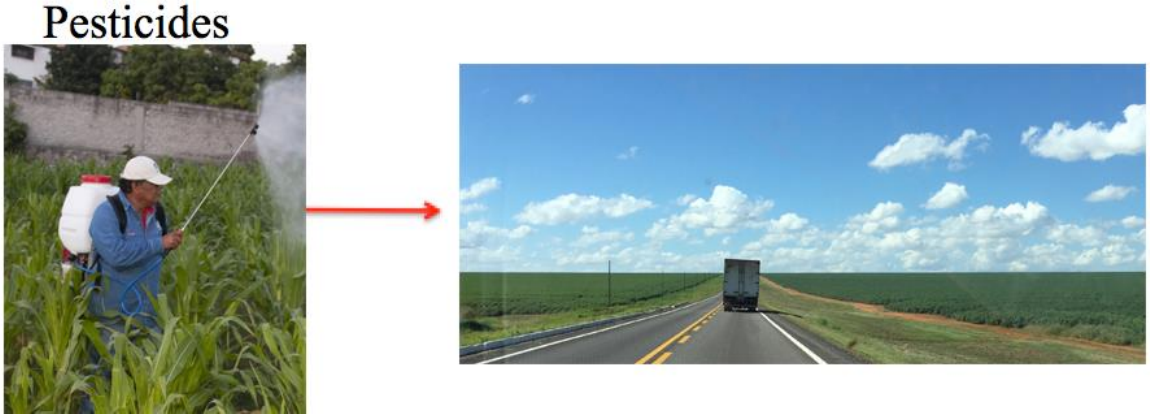
Biocontrol Microbes in Plant Protection: the Microbiology

1. ***Plant protection from insect attack***. Since the Green Revolution in the middle of the past century (1950-1960), the food demand of the growing world population has been met with a significant increase in crop productivity, due to agricultural intensification that depended on the use of high yield varieties of cereals and crops in association with employment of chemical fertilizers and other agrochemicals, including chemical insecticides, along with irrigation and mechanization. In this Green Revolution, chemical insecticides played a major role in reducing losses of crop yields due to insect pest attack. It has been estimated that up to 20 % losses of crops yields are due to insect attack.



Insect attack causes important losses in crop production. Insects may damage the final product of the crop, the foliage or the roots, resulting in important yield loses.

It is important to take in consideration that insects may affect plant productivity not only because they feed on plants but also because they transmit microbial plant pathogens, such as viruses and bacteria, that cause plant diseases and reduce crop yields. To protect plants from insects, insecticides are applied worldwide, including a variety of active compounds with different modes of action (disable or kill pests in different ways). Nevertheless, although chemical insecticides have been fundamental for assuring food production, it is now well established that most of them are also toxic to beneficial insects, such as pollinators, and to other non-target organisms, including mammals and plants. Moreover, most of these chemicals remain in the environment for prolonged periods of time affecting the environment in unpredictable ways. In the case of humans, it is well known that exposure to chemical insecticides causes different diseases such as cancer, lowered fertility, weakened immune responses and altered nervous system responses, among others. Thus, there is an urgent need to reduce the use of chemical insecticides in agriculture or to change for other chemicals that could be more easily degraded in the environment and that show lower toxicity to non-target insects and other organisms.

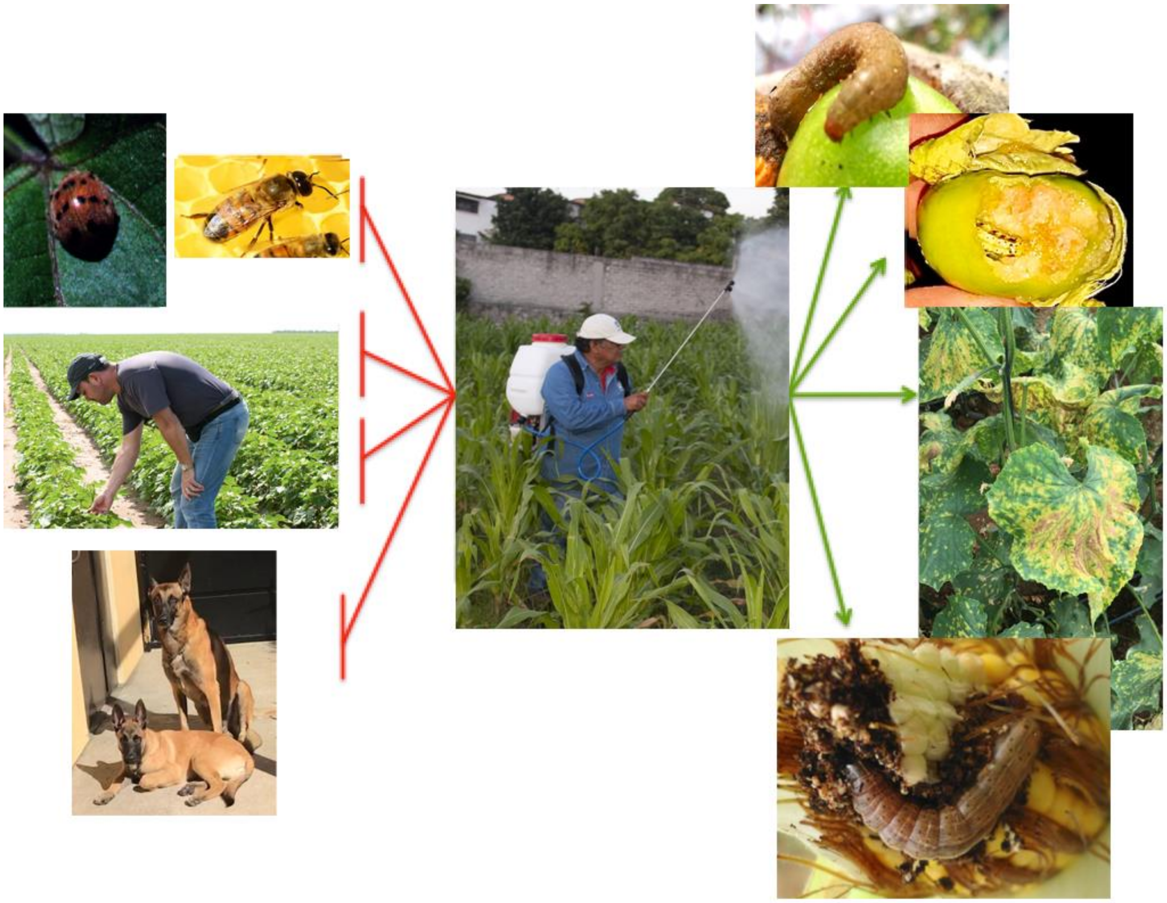


The use of chemical pesticides is fundamental to assure food production. However, they have important negative repercussions in the environment and on human health.

2. ***The concept of selective toxicity.*** A key property of medicines used to treat infectious diseases, and agrochemical pesticides used to combat crop pests, is *selective toxicity*, i.e. the toxicity of the medicine/agrochemical for the target pest – fungus, bacterium, insect, etc. – compared with its toxicity for non-target organisms, including humans. Ideally, the medicine/agrochemical should have a high selective toxicity, that is: effectively kill the pest but have no effect on other organisms. Selective toxicity is based on metabolic differences between the pest and non-target organisms: these differences offer possibilities of targets for such chemicals.

3. ***What chemicals are used in agriculture for plant protection?*** There are broadly two categories of insecticides: long-persistence–broad specificity insecticides, like organochlorine DDT, or low-dose–lower persistence–narrow specificity chemicals, like as neonicotinoids. With the onset of mechanization of agriculture, growers used long-persistence, broad specificity chemicals, like DDT, which was seen as a long-term solution for pest control in agriculture and for the control of mosquitoes that are vectors of important human diseases such as malaria, dengue, Zika, chikungunya, encephalitis and filariasis. However, a few years after DTT commercialization, it was recognized that DDT was extremely toxic to non-target insects and, more importantly, to humans, which led to its prohibition. Also, it is fair to say that DDT was shown to lose efficacy after some time, due to the fast evolution of resistance in the insect pests,

which reduced its toxicity and, in turn, prompted growers to apply increased amounts of this chemical in pest control, prior to its prohibition.



Novel insecticides need to be developed, that target only the insect pest without affecting beneficial insects, plants, animals or humans.

DDT was succeeded by other chemicals, like carbamates and organophosphates, as preferred insecticides for the control of insect pests. These in turn were succeeded by pyrethroids, which had the advantage of requiring lower amounts and being less persistent in the environment. Nevertheless, all these chemicals have recognized toxicity to non-target organisms and to humans. These days, compounds like emamectin benzoate and neonicotinoids, are widely used as low dose – low persistence insecticides. However, even these cause potential environmental and health problems, since it is known that they affect the nervous system of insects, humans, and other animals. Moreover, neonicotinoids are a principal suspect as the causal agent of honeybee hive decline around the world, through alteration of honeybee behavior. Interestingly, it has been suggested that the effect of neonicotinoids on honeybees is partially caused by the alteration of their microbiota. Thus, although agricultural practices moved towards the use of chemical compounds that show reduced hazard to the environment and to non-target organisms, current pesticides still exhibit selective toxicities that are not high enough. There is a growing need for new, effective insecticides with narrow specificities and low toxicities to other organisms, and low persistence in the environment,

4. ***What would be a perfect insecticide?*** A perfect insecticide should has the following properties:
   a. exhibit high selective toxicity, i.e. be a highly effective and specific compound that shows toxicity only against the target insect pest, without affecting other beneficial insects or other organisms,
   b. not persist in the environment for a prolonged period of time,
   c. not select resistance in the target insects,
   d. be inexpensive to produce and implement, such that its use is economically competitive in the marketplace.

5. ***Biocontrol agents can be highly specific.*** Microbial insect pathogens have been considered as interesting alternatives for the development of insecticides that show the characteristics described above. Insects can be the target of infectious diseases caused by different microbes, such as viruses, fungi or bacteria. The use of microbial pathogens for insect control, also known as *biocontrol*, has been recognized as an important alternative to reduce or substitute chemical insecticides. In contrast to chemical insecticides, microbial pathogens show high specificities for target pests and are non-toxic to beneficial insects and humans: they show high selective toxicity. A significant advantage of this is that, unlike the situation with agrochemical pesticides, which kill not only the target insects, but also their natural predators, which are usually other insect species that feed on the insect pests or that make use the pest larvae to growth their own offspring, biocontrol agents do not affect natural enemies of the insect pests. This means that target pests could be attacked by a combination of the biocontrol agents and by their natural enemies.

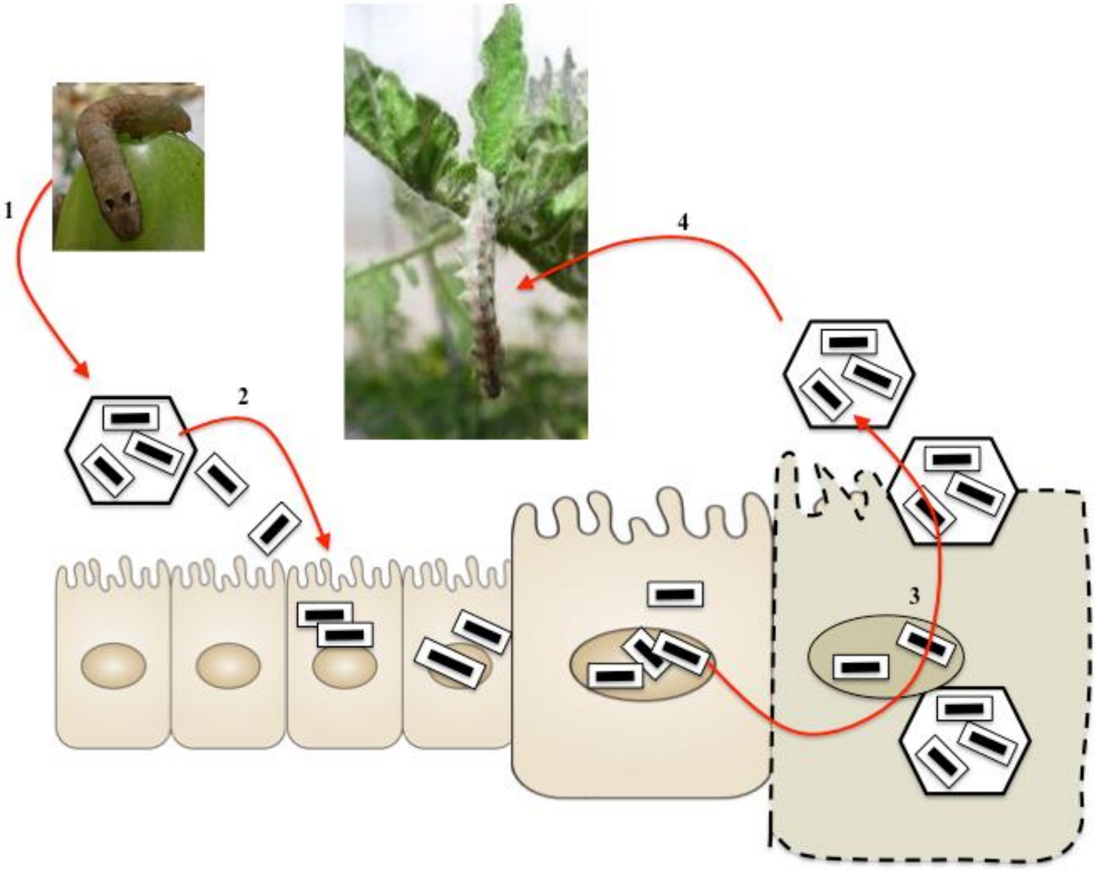
6. ***Use of biocontrol agents is limited at present.*** Despite the obvious advantages of biocontrol agents, their commercial use is limited to at most 2 % of the total market of insecticides. The reason is that most of these insect pathogens are mainly effective against young larval stages of the target insects, making necessary the use of precise timely application practices for efficient pest control. In addition, the aforementioned advantages of these bioinsecticides, namely their narrow specificity and low persistence in the environment, are taken as disadvantages by most growers, who still prefer broad spectrum insecticides that persist in the environment for long periods, reducing the number of insecticide applications and therefore lowering the labor cost associated with insecticide application.

7. ***Environmental awareness is driving demand for food produced without chemical pesticides.*** Food producers, however, are responding to growing concerns about the need to protect our environment and reduce human exposure to health-damaging chemicals. This trend is encouraged by governments, applying strict regulations on the amounts of chemical residues that are allowed in food products for human consumption and also for the export and import of many products. In addition, there is a growing market for organic agriculture, which produces vegetables and crops without the use of chemical pesticides. Insect control in organic agriculture is mainly based on the use of different bioinsecticides. Thus, there is a great demand

for the use of sustainable agricultural practices that are safe for the environment, safe for the growers and safe for the consumers. For the three types of insect pathogens (virus, bacteria and fungi), different commercial products have been developed and extensively applied.

8. ***Virus biocontrol agents.*** In the case of insect viruses, formulations with multiple virus species that show specificity to different insect orders have been developed. For virus infection to occur, larvae must ingest the virions, which then infect gut cells. Virus multiplication in the larval body results in the release of millions of virions into the environment, that can infect further insects of the pest population and result in an efficient control of the insect pest.

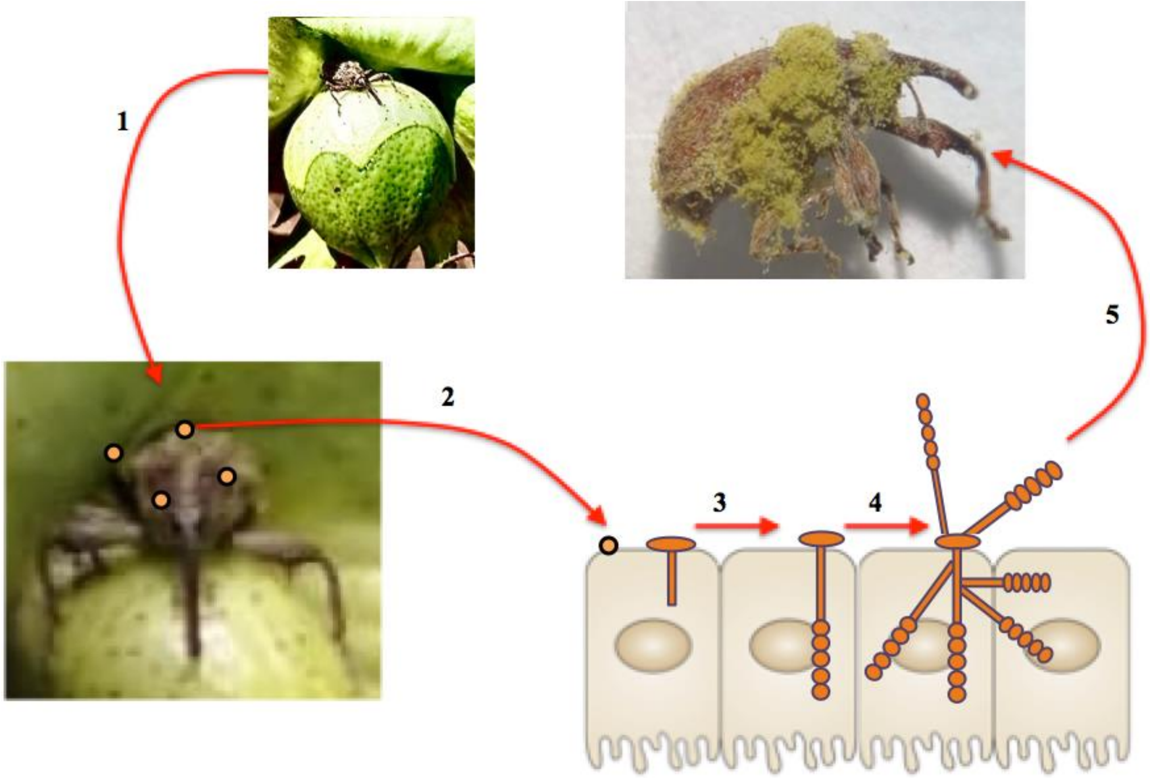
Viruses of the species *Baculoviruse*s have been applied successfully for crop production in many countries, such as the USA and Canada. In these countries, they have also proven to be highly effective against insect pests in forests and woodland.



Virus infection of insect pests. 1, larvae must ingest the virions deposited in plants. 2, virions infect gut cells and multiply inside to produce more virions. 3, new virions are released when midgut cells break down. 4, the final outcome is the insect death and release of virions into the environment.

9. ***Fungal biocontrol agents***. Commercial products based on different fungal species have been also developed. Infection occurs by penetration of the fungal hypha through the insect cuticle allowing the infection and spread of the pathogen in the whole insect body, finally killing the insect. However, fungal growth for insecticide production is inefficient and has only been partially achieved for a few species. Insect fungal pathogens that have been successfully

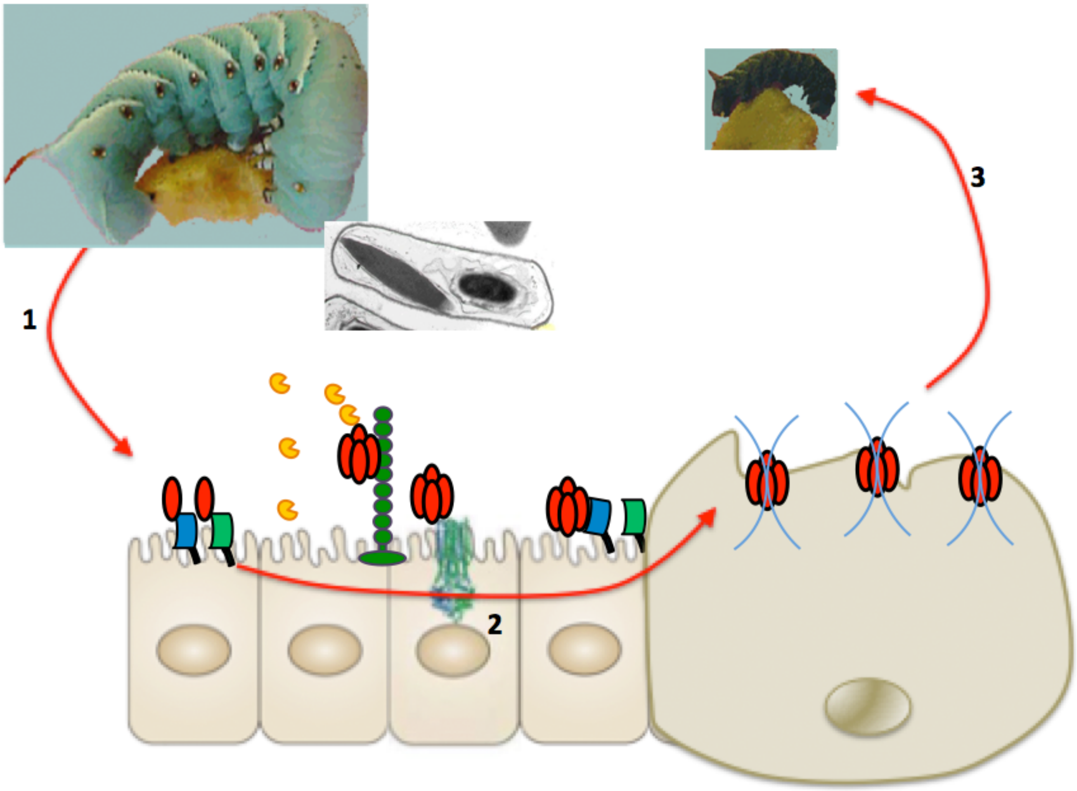
used for development of commercial insecticide formulations belong to the genus *Beauveria*, which infects a diverse range of insect species.



Fungus infection of insect pests. 1, the insect is in contact with the fungal spores. 2, the spores germinate and the fungal hypha penetrates the cuticle. 3, the infection spread inside the insect body. 4, pathogen infection can be observed in the whole insect body. 5, finally, the insect pest is killed.

10 ***Bacterial biocontrol agents.*** The most successful commercial bioinsecticides used worldwide are based on *Bacillus thuringiensis* (Bt) bacteria. Bt are Gram-positive soil bacteria that produce spores during their sporulation phase, when food sources are scarce. Bt spores are resistant to heat and desiccation and persist for a long period of time until a new food source is found allowing the spores to germinate and grow. During the sporulation process Bt produces large amounts of proteins, named Cry or Cyt, that accumulate in crystals inclusions in the mother cell compartment and are toxic for different insect orders, such as lepidopteran, coleopteran, dipteran, hymenoptera and nematodes. In addition, some Bt strains produce a different set of insecticidal proteins during the vegetative phase of growth named Vip toxins. These Vip proteins have been shown to be also highly effective against several lepidopteran crop pests.

In the case of the Cry proteins, the susceptible larvae ingest the spore/crystals produced by Bt strains, the crystals then dissolve in the larval gut and gut proteases activate these protoxins to yield the toxic activated toxin. Cry toxins are pore-forming toxins that insert into the membranes of the larvae gut cells, thereby causing the gut cells to die and break down, liberating their contents which provide nutrients for the spores to germinate which, in turn, invade the larvae, produce septicemia and kill the larvae. However, it has been shown that spores and septicemia are not necessary to kill the larvae for most crop pests, because the toxic activity of the Cry protein is sufficient.



Insect control with insecticidal Cry protein produced by the bacteria *Bacillus thuringiensis* (Bt). 1, larvae ingest the spore/crystals produced by Bt strains. Inside the larval gut, the insecticidal crystals are dissolved and gut proteases activate these protoxins to yield the toxic activated toxin that binds to specific receptors. 2, binding to receptors induces conformational changes in the toxin leading to oligomerization and pore formation in the cell membrane, which kills the cell. 3, the gut cells break down. The spores germinate, resulting in septicemia and killing of the larvae.

From the first Bt isolates that were characterized during the last century, it was recognized these bacteria have a high specificity for the different insects. For example, the Bt strains that show toxicity to lepidopteran larvae (butterflies) are not toxic to other insect orders, such as coleopteran larvae (beetles) or dipteran larvae (mosquitoes). Furthermore, the specificity of Cry proteins is remarkable. For instance, the Cry1A toxins, that are toxic to lepidopteran larvae, show toxicity only against limited number of lepidopteran species. The fall armyworm (*Spodoptera frugiperda*), which is a crop pest with worldwide importance, shows low susceptibility to Cry1A toxins but is highly susceptible to other Cry proteins such as the Cry1Fa toxin. Most Bt strains characterized produce more than one Cry protein giving the capacity to the bacterium to have a broader specificity.

Different formulations made with a few Bt strains have been commercialized so far for pest control. These formulations have been widely used for control of several lepidopteran, coleopteran and dipteran pests. However, their large-scale use as sprayable products is still limited, mainly due to their high production costs and the requirement for precise application practices, since Bt is mainly toxic to young instar larvae, and has to be applied with specific timing to achieve efficient control.

11. ***Transgenic plants.*** In addition to these Bt-based formulation products, the *cry* and *vip* genes that code for the insecticidal toxins have been expressed in multiple plants, thereby creating transgenic insect resistant crop plants. Indeed, so-called Bt-crops are the most successful global application of Bt for pest control, and have been shown to be highly effective in controlling specific insect pests. It is estimated that, compared with conventional non-transgenic crops, cultivation of Bt-crops is associated with 80 % lower levels of chemical insecticide usage.

12. ***The future of bioinsecticides.*** Although Bt-crops have been successful in reducing the use of chemicals in agriculture, there are countries that do not allow the use of this technology because of concerns of potential environmental risks of genetically manipulated crops. Thus, the development of alternative efficient bioinsecticides still has a great potential for agricultural use. Cry and Vip toxins expressed in Bt-crops are highly specific against their primary target pests, but they show no toxicity to other secondary pests and thus application of some chemical insecticides is still needed. In addition, as is the case with chemical insecticides, insect pests have also the potential to evolve resistance to these Cry or Vip protein expressed in Bt-plants, which will affect the future and continued use of Bt-crops in plant protection. The evolution of resistance by the pests prompted the search for new Bt strains with new *cry* genes that could help to control insects that have evolved resistance and that could be used in combination with other insecticidal genes in transgenic plants (known as pyramided plants). More than 700 different *cry* genes with different insect specificities have been identified and characterized so far in the search for new insecticidal genes for crop protection. However, the most important insect pests affecting main crops are susceptible to only a few Cry toxins and, in most cases, resistance to one Cry protein also promotes resistance to related Cry toxins with similar modes of action. Thus, researchers are looking for other bacterial species producing insecticidal proteins with distinct modes of action to known Cry toxins. *Pseudomonas chlororaphis*, *Pseudomonas mosselii* and *Alcaligenes faecalis* are recent examples of bacteria in which new insecticidal proteins not related to Cry or Vip proteins have been identified. Other soil microbes are expected to provide new insecticidal proteins with different modes of action to be used for efficient plant protection against insect attack and that would help in reducing the use of chemicals in intensive agriculture.

Relevance for Sustainable Development Goals and Grand Challenges

The microbial dimension for plant protection relates to several SDGs including:

- **Goal 2. End hunger, achieve food security and improved nutrition and sustainable agriculture.** The use of microbial insect pathogens for reduction of chemical insecticides on sustainable agriculture would achieve improved nutrition by diminishing the possibility of using hazard chemicals that could remain in the food. Also, the use of microbial insecticides would allow the use of other compatible control methods, since natural insect enemies are also susceptible to chemical insecticides.

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages (improve health, reduce preventable disease and premature deaths).** The reduction of chemical insecticides in agriculture by the increased use of bioinsecticides will reduce the risk of diseases such as cancer, and negative effects on reproduction, on immune responses and on the nervous system. People working in agriculture will benefit the most, since direct exposure to hazardous chemicals will be significantly diminished. However, all people that consume food produced by intensive agriculture will be also less exposed to toxic chemicals in their food.

- **Goal 6. Ensure availability and sustainable management of water and sanitation for all (assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems improve water and sanitation management).** The pollution of the environment by chemical pesticides includes pollution of water reservoirs. Reductions in the use of these toxic chemicals in agriculture, and replacement by biodegradable bioinsecticides, will reduce the pollution of water reservoirs.

- **Goal 7 Ensure access to affordable, reliable, sustainable and modern energy for all.** Renewable, non-fossil fuel-based forms of energy are key to sustainable development. Plant-based forms of renewable energy, however, suffer from major pest losses, which reduce their contribution and raise their costs. Current chemical insecticides are not environmentally friendly and thus not sustainable. Biopesticides are thus enablers of sustainable, plant-based renewable forms of energy.

- **Goal 14 Prevent and significantly reduce marine pollution.** Insecticides used on coastal farms enter and pollute the seas and poison wildlife. Replacing chemical insecticides with biodegradable, target-specific bioinsecticides will reduce the pollution of marine systems.

- **Goal 15. Halt biodiversity loss in terrestrial ecosystems.** Soil and plant-centric ecosystems harbor much planetary biodiversity. Insecticides reduce insect diversity and impact the health of other animals, so it is essential to reduce their application in the treatment of crop plants and replace them with target-specific biopesticides.

Potential Implications for Decisions:

1. ***Individual***
   a. Weighing up the various microbial and non-microbial factors and aligning them with personal convictions (do the personal positive health benefits outweigh the general environmental considerations?).
   b. Consume products from organic or from normal crop production (environmental footprint will be less by limiting the pesticides use for crop production?).
   c. Consume products from transgenic Bt-crops or from normal agriculture (environmental footprint will be less by limiting the pesticides use for crop production?).

2. *Community policies*
    a. Local environmental consequences (pollution of land and water bodies with chemical pesticides).
    b. Health costs associated with exposure to chemical pesticides in growers and consumers of contaminated plant products.
    c. Non-microbial parameters: support small organic producers and local businesses for local plant products.

3. *National policies related to plant protection*
    a. Health care economics of poisoning by exposure to chemical pesticides.
    b. Environmental pollution and bioremediation.
    c. Ensure safe food and water supplies.
    d. Recovery of polluted lands for future crop production.

Pupil participation

1. ***Class discussion associated with plant protection***

2. ***Pupil stakeholder awareness***
    a. Plant protection from insect attack with chemical insecticides or with biocontrol agents has positive and negative consequences for the SDGs. Which of these are most important to you personally/as a class?
    b. Can you think of anything to enhance the use of bioinsecticides to reduce the use of chemicals in agriculture?
    c. Can you think of anything you might personally do to reduce the environmental impact of chemical pesticides in agriculture.

3. ***Exercises***
    a. Most of our food supplies come from intensive agriculture that uses chemical pesticides for crop production. What other alternatives exist for assuring food supplies with reduced use of pesticides for crop production?
    b. Bioinsecticides are safe, but require precise formulations to protect these products from sun light and precise application practices to achieve efficient pest control. Can you think of anything that might improve their efficacy?
    c. What other microorganisms could be good sources of proteins with insecticidal activity? Why?
    d. Which might be the most promising places for identification of such microorganisms?

The evidence base, further reading and teaching aids

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Glossary

Alcaligenes faecalis, a species of Gram-negative, rod-shaped bacteria commonly found in the environment. ***Bacillus thuringiensis***, a Gram-positive rod-shape bacterium that during its sporulation phase produces a parasporal inclusions formed by insecticidal proteins such as Cry, Cyt and Vip

Baculovirus, pathogenic viruses that attack insects and other arthropods

Bacteriophages, type of virus that infects bacteria

Beauveria, a genus of asexually-reproducing fungi. Several species are insect pathogens

Beneficial insects, useful insects that do not damage plants or human health, such as spiders, are predators of insects, parasitic insects, pollinators, bees, honeybees, butterflies, and insects that are food for other organisms

Bioinsecticides, organisms including bacteria, viruses, and fungi highly specific for certain insect pests and may persist in the environment

Bioremediation, a branch of biotechnology that employs the use of living organisms, like microbes and bacteria, in the removal of contaminants, pollutants, and toxins from soil, water, and other environments

Bt-crops, transgenic crops that produce the same toxin as the bacterium *Bacillus thuringiensis* in the plant cell, thereby, protecting the crops from pests

Carbamates, a category of organic compounds that is formally derived from carbamic acid

Chemical insecticides, chemical substances toxic to insect pests

chikungunya, disease caused by Chikungunya virus that is spread to people by the bite of an infected mosquito.

Coleoptera, an order of insects that includes beetles

Crops, plants or products that can be grown and harvested extensively for profit or subsistence.

Cry toxins, a large family of crystalline toxins produced by *Bacillus thuringiensis*. Composed of three domains and produced as crystal inclusions during sporulation phase of *Bacillus thuringiensis*

Cyt toxins, constitute a smaller, distinct group of crystal proteins produced by *Bacillus thuringiensis* with insecticidal activity against several dipteran larvae, and display cytolytic activity against red blood cells

δ-endotoxins, are pore-forming toxins produced by *Bacillus thuringiensis* species of bacteria.

DDT, Dichlorodiphenyltrichloroethane is an organochlorine compound

dengue, A mosquito-borne viral disease occurring in tropical and subtropical areas

Diptera, an order of insects that includes flies and mosquitoes

Encephalitis, inflammation of the brain

Enzymes, proteins that act as biological catalysts accelerating chemical reactions

Epizootic, a disease that is temporarily prevalent and widespread in an animal population.

Filariasis, A tropical, parasitic disease that affects the lymph nodes and lymph vessels

Gram-positive, bacteria are bacteria that give a positive result in the Gram stain test, which is traditionally used to quickly classify bacteria into two broad categories according to their cell wall structure.

Green Revolution, initiatives evolved from research technology transfer that increased agricultural production worldwide, beginning most markedly in the late 1960s

Horizontal gene transfer, is the movement of genetic material between unicellular and/or multicellular organisms other than by the transmission of DNA from parent to offspring (reproduction).

Human diseases, any condition that causes pain, dysfunction, distress, social problems, or death to humans

insect cuticle, the outer covering of the insect and is its exoskeleton to which the muscles are attached

Insect pathogens, pathogens that cause disease in insects

Insect pests, insects that have adverse and damaging impacts on agricultural production, market access, the environment, and human health

Insect specificity, specificity of activity limited to insects

Lepidoptera, an order of insects that includes butterflies and moths

malaria, is a serious disease caused by a parasite transmitted to humans by the mosquito *Anopheles* spp.

Metabolic pathways, routes and networks of chemical reactions in a cell that build and break down molecules for cellular processes.

Microbe insect pathogens, microorganisms (bacteria, fungi, microsporidia, virus) that are able to kill insect pests

Microbiota, community of microorganisms found in a particular ecological niche forming microbial ecosystems

Neonicotinoids, a class of neuro-active insecticides chemically similar to nicotine

Organochlorine, organic compounds that contain at least one covalently bonded chlorine atom

Organophosphates, organophosphorus compounds with a central phosphate molecule with alkyl or aromatic substituents.

Parasitoid, an insect whose larvae live as parasites that eventually kill their hosts (typically other insects).
Plant pathogens, an organism that causes a disease on a plant
Plant protection, area of agriculture dealing with preventing losses caused to crops by diseases, pests and weeds
Plasmids, small, extrachromosomal DNA molecules within a cell that are physically separated from chromosomal DNA and can replicate independently
Predators, wild animals that hunt, or prey on, other animals.
Pseudomonas chlororaphis, is a Gram-negative bacterium used as a soil inoculant in agriculture and horticulture.
Pseudomonas mosselii, is a Gram-negative, rod-shaped, bacterium
pyramided plants, plants containing two or more dissimilar *Bacillus thuringiensis* toxin genes in the same plant
Pyrethroids, an organic compound similar to the natural pyrethrins, which are produced by the flowers of pyrethrums
Soil bacteria, Bacteria found in the soil.
Spodoptera frugiperda, also known as the fall armyworm, is a species in the order Lepidoptera
Sustainable agriculture, agricultural practices meeting society's present food and textile needs, without compromising the ability for current or future generations to meet their needs.
Transgenic plants, plants into which one or more genes from another species have been introduced into the genome, using genetic engineering processes
Vip toxin, Another class of *Bacillus thuringiensis* insecticidal toxins that are produced during the vegetative growth phase
Zika, A disease caused by Zika virus that is spread through mosquito bites.