Biological control of insect pests destroying crops: from Louis Pasteur to GMOs

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Introduction

It is a great pleasure and an honour for me to have been invited to speak at this prestigious university. I am very grateful to Laure Schwartz, who happens to be my niece, to have organized this conference.

The topic chosen for this conference fits very well, it seems to me, with the general subject of this symposium, which deals the question of food and the culture of rice and cereals in Japan. Indeed, although this conference is not meant specifically for a Japanese audience, it deals with a worldwide problem, that of the damage caused to crops by insects, and the means that have been invented by mankind to control these insects. This will lead us to a discussion of the question of transgenic plants, often designated as GMOs (Genetically Modified Organisms)

I do not know what the situation in Japan is but, in Europe, a controversy has been raging for more than 20 years about these GMOs. Although these have been widely cultivated for the last ten years or so in a number of countries outside Europe, they are opposed by many Europeans. I will come back to this controversy at the end of this conference. Before that, what I would like to do is to show you that the making of a particular class of genetically modified plants, those that are resistant to insect pests, is the end point of a very long process of development that began at the dawn of humanity, when humans first became farmers.

The fight against insect pests started very long ago

But first, let's make one thing clear: not all insects are the enemies of humans. Indeed, many make a valuable contribution to agriculture as pollinators and, more generally, are essential elements in the ecological balance of the planet, not forgetting, of course those that actually work for us, like bees and silk worms. However, some insects are real enemies, either because they spread serious diseases (such as malaria, yellow fever or Japanese encephalitis), or because they feed on crops or forests, causing extensive damage. It is this second type of pest that we will consider here.

Ever since humans first began to cultivate plants, insect pests have been among the most insidious and dangerous of our enemies. They have been responsible for countless famines throughout history. Everyone has heard about the devastation caused by locust invasions. For instance, even though most of you are probably not Christians or Jews, you probably know of the Bible, and have heard the story of Moses whose people, the Jews, was retained in Egypt by the Pharaoh. This was about 1500 years before Christ. Moses went to see Pharaoh and asked him to let his people go. Since Pharaoh refused, Moses, inspired by God, threatened him to send terrible plagues over Egypt. Successively ten such plagues were thus sent over Egypt. And here was the 8^{th} plague. Similar invasions of locusts have repeatedly occurred on all continents, even sometimes in Europe, including a recent example in France. These invasions may become increasingly frequent as a result of climate change.

The destruction of the harvest by many types of insect, at adult or larval stages, has always been and remains a cause of major agricultural losses. Insects may directly attack the plant in the field or the harvested crop product, particularly cereals in warehouses. This is the case, for example, for rice — an essential source of food for much of the world's human population, particularly in this part of the world — for which about 30% of potential yield is thought to be lost to insects. It is therefore not surprising that humans began to fight these enemies in very ancient times.

In 70 AD, for example, the Roman scientist Pliny the Elder wrote that a green lizard should be suspended from the top of apple trees to protect them against maggots, whereas a fish should be used in the same manner to protect against ants. Through the ages, a multitude of solutions of this type have been proposed. However, as these solutions were often far from effective, many farmers preferred to trust in divine justice. In countries of Christian religion, insect pests were considered to be the agents of the devil. In France, for instance, bishops have been known, during the fifteenth century, to call the caterpillars before them, to provide them with an advocate for their defence, and to excommunicate them, demanding that they leave the area. Such appeals to divine justice persisted for several centuries. However, since even these extreme measures did not seem to have been entirely satisfactory, farmers rapidly began to make use of chemical weapons.

Introduction of chemical insecticides

Two thousand years ago, a powder prepared from pyrethrum flowers (a relative of the chrysanthemum) was already being used as an insecticide by the Chinese, who had noticed that these flowers were never attacked by insects. Since that time, and in various parts of the world, preparations of highly diverse composition but generally of uncertain efficacy have been used. The substances used have included ash, sawdust, the urine of domestic animals, limed water or decoctions of plants. In the middle of the 19^{th} Century, the products of physical chemistry, such as carbon sulphides or mixtures of copper salts, were widely used against *Phylloxera* that attacked the vineyards. Similarly, products based on arsenic and sulphur, such as copper acetoarsenite was used against Colorado beetle, which destroyed potato crops.

However, the chemical insecticide industry did not really begin to take off until the middle of the 20^{th} Century, following the discovery of the insecticidal properties of DDT (dichloro diphenyl trichlorethane) by the Swiss scientist, Paul Muller. For several years, DDT was hailed as a miraculous and absolute weapon against insect pests. It played also a major role in combating the insect vectors of diseases, including the anopheles mosquitoes responsible for malaria transmission.

Unfortunately, various problems rapidly appeared, including ecological problems (effects on non-target species), a lack of efficacy (emergence of resistance in insects) and problems of possible toxicity in humans, due to residual traces of the product both in the plants treated and in the animals feeding on them. Many other molecules were subsequently released onto the market, with a view to overcoming one or several of these problems. Some of these molecules are among the insecticides still in use today.

However, despite these efforts, the chemical insecticides in widespread use remain toxic products, dangerous to the farmers using them and with largely uncontrolled long-term effects on the environment.

For this reason, biological methods of controlling insect pests have long attracted the attention of scientists.

Louis Pasteur, promoter of the biological control of insects

We often forget that Pasteur made a seminal contribution to the endless war against insects. The insects on which Pasteur first focused his attention were not likely to devastate crops; they were silk worms. During the first half of the 19^{th} Century, silk production developed rapidly in France, with annual production expanding to reach one tenth of the world's total. However, towards the middle of the 19^{th} Century, a variety of devastating silk worm diseases emerged in southern France, threatening the entire silk industry. Pasteur was asked to investigate these diseases.

After five years of intensive study, Pasteur established that silk worms were susceptible to two highly infectious diseases, called "pébrine" and "flacherie". Pébrine is caused by a parasite, whereas Pasteur concluded that flacherie was cased by a bacterium. He then went on to propose solutions to prevent the dissemination of these diseases, thereby saving the silk industry. These solutions — including "grainage", which allowed one to separate the eggs of contaminated females from those of healthy females — are still used in silk-producing countries, including Japan, where I was told that they are enshrined in a law.

In 1872, two years after the publication of his famous work "Studies of the Diseases of Silk Worms", Pasteur attended a "viticultural and sericicultural congress" in Lyons, were he heard a talk about the damage to vines caused by *Phylloxera*. At the end of this presentation Pasteur declared his view that, like silk worms, *Phylloxera* might be susceptible to pébrine. He suggested putting silk worms with pébrine into a glass of water and then pouring this water over the infested vine in the hope that the pest insect (*Phylloxera*) would catch the disease (pébrine). He hypothesised that this would provide a means of infecting the females, which would then transmit the lethal disease to their progeny.

A few years later, in 1880, probably after having learnt that pébrine had no effect on *Phylloxera*, Pasteur developed this idea further. He applauded the discovery and use of insecticides, which had already proved highly effective, but pointed out that their effects were local. He put forward the idea that a main hope for triumphing over a life form with as strong a reproductive capacity, like *Phylloxera*, was to exploit another life form with even greater reproductive capacity. He suggested that like all living species, *Phylloxera* must have its diseases, parasites and natural causes of destruction, which could then be used to control it.

The idea underlying biological control is there, clearly expressed. At exactly the same time, in 1973, the American entomologist John Lawrence LeConte, also proposed the use of infectious agents, including fungi in particular, to combat insects.

The use of entomopathogenic bacteria

Although Pasteur did not himself attempt to use microbes against insects, others, including some of his followers, did. One of those who did was Felix d'Hérelle, better known for his discovery, simultaneously with Twort, of bacteriophages — viruses that infect bacteria.

In 1910, whilst working in Mexico, d'Hérelle witnessed the terrible damage to crops inflicted by swarms of migrating locusts. This led him to look for a natural bacterial disease of these insects. He isolated a bacterium responsible for epizootics in locusts and used it, with some success, to control the insect population.

The bacterium isolated by d'Hérelle was only moderately effective against insects, but this work may have inspired other scientists. One such was the German Ernst Berliner who, in 1911, isolated a pathogenic agent from the larvae of diseased insects found in flour. In 1915, he named this agent *Bacillus thuringiensis*. This bacterium was later shown to produce a toxin highly active against insects, with different strains producing toxins active against different types of insects. This bacterium is among those that produce spores, i.e. a very resistant form when they are starved. The toxin is produced as a crystal during bacterial sporulation.

Work at the Pasteur Institute during the 1970's demonstrated that this toxin actually consisted of several similar proteins active against the intestinal cells of the larvae of susceptible insects, preventing these larvae from feeding and developing. The production of these toxins must be advantageous to the bacterium, providing it with an ecological niche – the dead larvae- for growth and then dispersal in natural conditions.

At the end of the 1950s, *Bacillus thuringiensis* (Bt) and its toxins were beginning to be used as biological insecticides. Their specificity and lack of toxicity to humans were among their principal advantages.

Scientists from the Pasteur Institute, the French National Institute of Agricultural Research (INRA) and others have demonstrated the efficacy of large-scale applications of various strains of *B. thuringiensis* for protecting cereal and fruit crops and forests against insect pests.

However, these biological insecticides do not persist for a long time after application, impairing their efficacy. With the development of genetic engineering, it has become possible to identify and to isolate the genes encoding these toxins from various strains of Bt and to introduce them into plants.

Genetically modified plants producing toxins

So how do you go about introducing these toxin genes into plants?

Like all other proteins of living cells, the toxins of these bacteria are encoded by genes — segments of the bacterial chromosome. Genes are composed of DNA, a long, linear polymer composed of four chemical compounds (the nucleotides A, T, G and C), the order of which is essential. Indeed, through the workings of a complex machinery that I will not describe in detail here, this order determines the order of another set of compounds — amino acids — in the protein encoded by the gene. The sequence of nucleotides (or bases) differs between genes, so the sequence of the amino acids they encode also differs. Finally, the order of the amino-acids in a protein determines its properties (in our case, the toxicity of the Bt toxin).

The first step is therefore to break open the bacteria that produce the toxin, to cut up the DNA they contain and to isolate a fragment carrying the gene encoding the toxin. I do not intend to describe the details of the methods used to recognise this fragment. However, I would like to say a little about the next step, which consists of introducing this fragment of DNA into a plant cells. One of the principal methods used for this step involves a phenomenon demonstrating that Nature did not wait for humans to start genetic engineering.

We will now take a little detour and consider a phenomenon that many of you may have observed yourselves: the existence of plants with bizarre outgrowths, sometimes bearing branches or roots growing out in all directions. This phenomenon resembles a sort of plant cancer. This apparent similarity to cancer has excited considerable interest. In some cases, this outgrowth, which is known as crown gall, is caused by a bacterium, *Agrobacterium tumefaciens*. Like many other bacteria, *Agrobacterium* has not only a chromosome bearing most of the genes encoding the proteins it needs, but also a small extra chromosome, known as the Ti plasmid (Ti for tumour-inducing). This plasmid has a very surprising property. It contains a fragment of DNA (the transfer - or T-DNA) that can be spontaneously transferred from the bacterium to a plant cell. Once this DNA fragment reaches the inside of the plant cell, it inserts into one of the plant's chromosomes and the genes that it contains (12 in total) begin to behave as if they were plant genes. The inserted DNA confers two new properties on the plant cell: the synthesis of two plant hormones triggering uncontrolled growth of the cells leading to the formation of this tumour-like structure on the plant and the production of a molecule specifically for the nutrition of *Agrobacterium tumefaciens*. So, by this ingenious mechanism, the bacterium forces the plant cell to multiply and to synthesise food for it!

As you can see, this process is really a type of "natural" genetic engineering, because it involves the transfer of bacterial genes to a plant. It has been as useful to scientists as it is to the bacterium, as it inspired them to make use of this phenomenon to introduce other genes into plants. They extracted the bacterial plasmid and removed most of the T-DNA fragment, leaving only the parts necessary for insertion into the plant chromosome. The genes removed could then be replaced by the genes they wished to study, including the genes encoding Bt toxins. The modified plasmid was then reintroduced into *Agrobacterium*, which was then placed in contact with the plant cells into which the scientists wished to introduce the toxin genes.

The final steps in the process involve the selection of cells containing the desired genes and the regeneration of plants from these cells. This final step is possible because plant cells, unlike their counterparts in animals, can be easily used to generate an entire new plant.

The transgenic plants produced in this way can produce their own toxins to kill the insect larvae that attack them. The first success of this technique was the production, in 1987, of tobacco plants resistant to lepidopteran larvae. Bt toxin genes were subsequently introduced into various important crop species, including maize, providing protection against the European corn borer, one of the major pests of maize and a cause of considerable economic losses in untreated crops. Bt toxin genes have also been introduced into other major crop species, including cotton, soybean and potato.

For Pasteur and all the other scientists who have tried, over the centuries, to protect crops against insect attacks, this looks like the final victory. It represents the ultimate in biological control — providing plants with weapons to defend themselves. It consumes less energy and is more environmentally friendly and, probably, more effective than any type of insecticide yet developed, whether chemical or biological in origin. Many countries in which these issues are considered important have moved towards the cultivation of transgenic plants, often combining insect resistance with herbicide tolerance, making it possible to apply the herbicide in question to eliminate weeds without affecting the growth of the crop plant. In 2007, the total area under genetically modified crops (GMOs) had reached 114 million hectares, 12% more than in the previous year. In the United States, for example, about 60% of the 250 million tonnes of maize produced each year is genetically modified. However, the use of this technique is a major source of controversy in Europe, where the introduction of GMOs has come up against very strong opposition.

The controversy surrounding GMOs

Why is there so much opposition? Various arguments have been put forward opposing the cultivation and sale of GMOs; unfortunately, the debate is complicated by the amalgamation of these arguments such that the true reason for opposition is obscured. There are essentially five types of argument against the culture and/or sale of GMOs:

- There may be health risks associated with the consumption of GMOs
- There may be environmental risks associated with the cultivation of GMOs
- The production of GMOs is "unnatural" and should be rejected as such
- The sale of GMOs poses economic problems
- GMOs are not useful.

What should we make of these arguments?

GMOs may present a risk to health

This argument is undoubtedly the most widespread among the public but is probably the least well founded.

Firstly, of course, scientists avoid introducing genes encoding products associated with risks to human health, however small, into plants destined for human consumption.

Secondly, before being released onto the market, GMOs destined for human consumption are subjected to extremely severe testing to ensure that they are neither toxic nor allergenic. These tests, if applied to the "normal" products long consumed by humans, would undoubtedly lead to prohibition of the consumption of many of these products. This would clearly be the case, for example, for potatoes, the skin of which contains a toxin, and kiwi fruit, which causes allergy in many people.

Thirdly, GMOs have been consumed in large quantities by the populations of many countries including those of North America over the last 10 years or so and NO deleterious effect on health resulting from the consumption of these products has ever been reported.

GMOs may present a danger to the environment

It has been suggested that the genes introduced into crop plants could be transmitted to wild plants, including undesirable plants in some cases, potentially causing an ecological disaster. This argument needs to be examined on a case-by-case basis. However, it should be borne in mind that, for such a phenomenon to occur, the gene must be transmitted from the crop plant to the wild plant at a detectable frequency and must confer a selective advantage on the wild plant (it would probably be eliminated from the wild population if this were not the case).

The probability of gene transmission from a crop species to a wild species depends on the presence in the environment of the crop of wild species related to the crop. For example, for maize, the probability of transmission to a wild plant is close to zero in Europe, where there is no closely related species. However, the probability of transmission is much higher in Central America, where the ancestors of cultivated maize are still found.

The question of selective advantage is all too often overlooked. A gene conferring herbicide tolerance undoubtedly confers a selective advantage on the crop plant if the crop is treated with the appropriate herbicide. However, it would confer no selective advantage on a wild plant in an area in which this herbicide is not used. Such a wild plant rendered accidentally transgenic would have no chance of developing more rapidly than its congeners (otherwise similar plants) and, therefore, no chance of causing an ecological disaster.

Certain other characters, such as resistance to insects or to viruses, for example, might pose problems and require more careful examination; this is already required as part of the licensing process.

GMOs should be banned because they are "unnatural"

GMOs are monstrosities that humans should avoid producing. In the mind of many people, GMOs are similar to the numerous chimeras that many artists have represented in the past. This argument against genetic engineering came to the fore at the time of its early development, in the middle of the 1970s. It is a matter of personal conviction and principles. We have to respect this point of view. However, we should remember that none of the crop plants that we currently consume is natural, all having undergone large numbers of genetic modifications. The plants and animals destined for human consumption today only remotely resemble their natural ancestors as they existed in the wild. They are the result of a multitude of mutagenesis and selection events.

GMOs pose economic problems

This seems to me to be the crux of the question. The principal criticism is that GMOs provide large multinational companies with considerable leverage over farmers and in particular can lead to companies having exclusive rights to certain seeds.

One of the potential consequences of this situation is a lessening of genetic diversity if only GM varieties are cultivated. There is clearly an economic dimension to the question of GMOs and our attitude to this question results largely from our own political convictions. It is clear that the Americans would be the biggest winners if the market for GMOs were to develop in Europe today. One can adopt a position of being for or against this. The question of possible decreases in the genetic diversity of crop species is a real issue. This trend is already well established and might be reinforced by GMOs.

GMOs are not useful

Having considered the possible risks of GMOs, we should also briefly consider the potential or actual benefits of these plants. Indeed, even if the risks are very small, why should even small risks be taken if the benefits are also very small, or even non-existent? Looked at from another angle, do we worry about the possible risks associated with heating food in a microwave or of eating an exotic product that we have never eaten before? The answer is "no", because the benefits are clear.

For farmers, the GMOs currently available present clear advantages. In principle, cultivating plants resistant to insects decreases or eliminates the need to treat the crop with insecticides, thereby saving both money and time. If growing GMOs was of no benefit to farmers, GM crops would clearly never have been as successful as they are in countries like the US. However, for consumers, these advantages are not obvious, particularly as we already produce more food than we consume in Europe. Consumers would be much more sensitive to the possible benefits of GMOs if these could be shown to be beneficial to health. Have there been any advances in this domain?

As we have seen, the GMOs currently on the market were generated to facilitate agricultural practice rather than to provide health benefits. However, these GMOs could have health benefits. The cultivation of crops resistant to insects should reduce the use of insecticides, which are potentially toxic. In general, the accumulation of pesticides in the environment presents a genuine problem and any attempt to reduce their use should be seen in a positive light. Resistance to insects may have the additional advantage of rendering the plant more resistant to moulds, because moulds often penetrate plant tissues via wounds caused by insects. Many of these moulds produce mycotoxins, which are highly toxic and dangerous to human health.

However, these potential advantages have not yet been demonstrated entirely convincingly, although a decrease in insecticide use has been demonstrated in certain cases. In the US, for example, the decrease in insecticide use in cotton farming between 1995 (the year preceding the introduction of Bt varieties) and 1999 has been estimated at 1200 tonnes, corresponding to 14% of the total amount used. However, these decreases are not always as clear as one might hope. The benefits due to decreases in mycotoxin consumption are undeniable, but difficult to quantify precisely.

We have dealt only with the case of transgenic plants currently on the market. It should not be forgotten that scientists and industrial companies are now developing “second-generation” GMOs, which should have more tangible advantages for consumers. For example, drought-tolerant varieties are being developed and may prove essential in the near future, when water resources are likely to become a major ecological issue on our planet. Should we reject out of hand a technology that humanity may greatly need tomorrow?

Conclusion

When we consider the current controversy surrounding GMOs, we end up asking ourselves why the emergence of these crops in the landscape was so sudden. I hope to have shown you that, at least in the case of GMOs designed to be resistant to insects, the development of these crops is the end point of a long process, which began many centuries ago. Also, I hope to have clarified somewhat the debate concerning the acceptability of theses GMOs and shown you that this technology, although it may have some undesirable consequences, mainly from an economic point of view, may offer a great potential for the production of food in a world where food, precisely, is becoming a rarity.

One should be aware, in particular that it is impossible to abandon both GMOs and insecticides if we are to feed the world’s population, which continues to grow.

I would like to end with a little publicity for my institute, the Pasteur Institute, which does not work on transgenic plants, but which does use genetically modified micro-organisms and cells to advance life sciences for the benefit of human and animal health.

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