By Paul Mamot & Jonas Blatter

BT-corn – genetically modified maize

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1.Preface:

We, Paul Mamot and Jonas Blatter, chose to work on genetically modified crops and especially the Bt-corn, because the field of GMO's in the food industry is still rather new. We both didn't fully understand the practice, so we began to ask questions. How does one modify the seeds in the lab? How exactly does it protect the plant from foreign intruders? But most importantly, what are the risks and downsides and equally the benefits of genetically modified crops? For this last question, we really couldn't imagine a possible answer, as we had no clue on this part of the topic. Therefore, this was our main concern and one of the key aspects, which led to our chosen topic.

2. Introduction:

Food supply was and is up to this day one of the main challenges of human kind. Therefore it is normal for us to always seek methods that increase our food supply. We have done and will always do that. As far as agriculture goes back, humans have always "genetically modified" their crops by selecting and breeding the crops, that resulted in the highest yield. Today, this whole process is happening in the lab, which is less time consuming and the benefits are much more controllable. The whole GMO (Genetically modified organisms) industry is a relatively new sector in science. The first GMO's were introduced in the 90s. Since then, there has been a lot of speculation in society. People are not really sure, what to think about it. In Europe, companies are required by law to label their products, if they were made with the help of GMO's. But in the USA and Canada, this is no common practice. The „agricultural business" literally fights against this transparency with their customers and influences decisions in congress about this topic. The result is an ongoing debate or as to say: a big question mark on the side of the society, which obviously is not educated in this area or just to a certain extent. If now paired with the intransparency of the producers, the consumers are left with incomplete knowledge and misleading labeling.

At this point in time, genetically modified crops have been used for a good amount of time. Therefore scientists have acquired a decent amount of data. Nowadays, the first long-term successes or failures reveal themselves. In the recent years, scientists took the acquired data from the past decades and tried to draw a conclusion, especially on whether the Bt-corn is safe for humans in the long run or not and who exactly benefits from the GM crop, both in terms of biology and economics. For example, this research team from the UMD (University of Maryland, USA) pulled together data from the past forty years and checked on the effects of Bt-corn onto so-called "offsite crops" in North America. And the results are impressive. As Bt-corn is obviously secured against pests like the European corn borer (*Ostrinia nubilalis,* see picture to the right), other plants, for example the previously mentioned "offsite crops", are not. Pests as the corn borer are not solely harmful to corn but also to numerous other crops. And that's

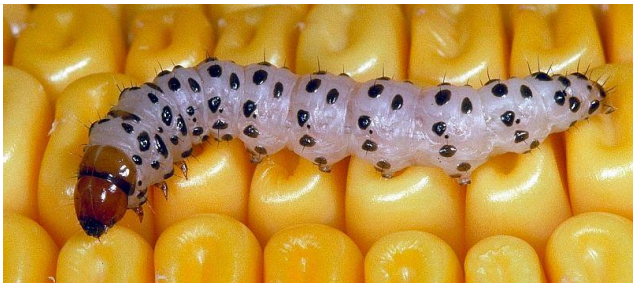

Figure 1: Ostrinia nubilalis

where the Bt-corn comes to help. Due to the modified DNA of the corn, it can kill the corn borer and therefore reduce the overall population of the pest, which leads to less damaged corn fields, but also less damage to the surrounding fields, the "offsite crops". The results are quite astonishing, as the population of the corn borer was suppressed up to 90% in the areas of examination. And according to the research team, this huge decrease in pests can be traced back to the Bt-corn.

Alternatives or in other words, variety and diversity have always been important and key factors for an optimal yield. As we all know, too much of one crop, animal or the like, makes it fairly easy for pathogens or natural enemies. And as well with Bt-corn, variety is important, because otherwise, there will develop pathogens and insects which are resistant to the *Bacillus thuringiensis*. In terms of alternative treatments to Bt-corn, a lot of research has been done. But one alternative in particular is on the uprise. And it is also surprisingly similar. Instead of using BT *(Bacillus thuringiensis)*, the use of another bacterium has captured the attention of researches. *Photorhabdus luminescens* - a bacterium which creates a family of highly potent insecticidal proteins that are capable of killing a wide range of insects, such as cockroaches, boll weevils, corn rootworm and many more. All the way back in 1998, the first results from the research on this new bacterium was released in a journal by scientist from the University of Wisconsin in collaboration with the Indianapolis-based company "Dow Agrosciences". It is, as mentioned before, a potent pathogen, as the bacteria lives inside the gut of nematodes. Once inside a host, the bacteria release itself and kill the nematode. The bacteria also start reproduction and inevitably kill the insect. What is left of the host is its glowing body. Hence, the name of the bacterium - *Phototrhabdus luminescens.*
"We have found at least seven different types of insecticidal proteins produced from these soil-borne bacterium carried by the nematodes," says the research company Dow Agrosciences. "These bacteria carrying nematodes are very prevalent and I'm sure there may be more unique and useful strains to uncover. We're just scratching the surface and we're trying to better understand how they work since they're a diverse group of bacteria — more complicated than Bt toxins."
The alternative seems promising for the future of genetically modified crops, but it is just not yet ready for a large-scale production.

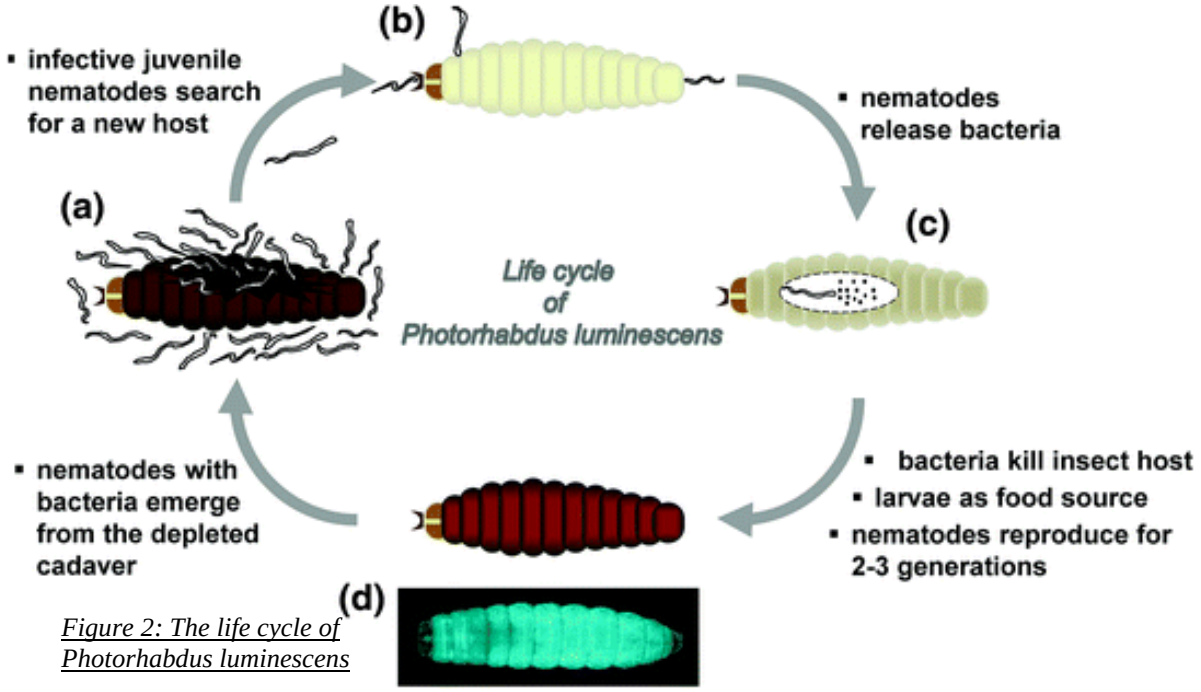

Figure 2: The life cycle of Photorhabdus luminescens

3. Description of engineering technique:

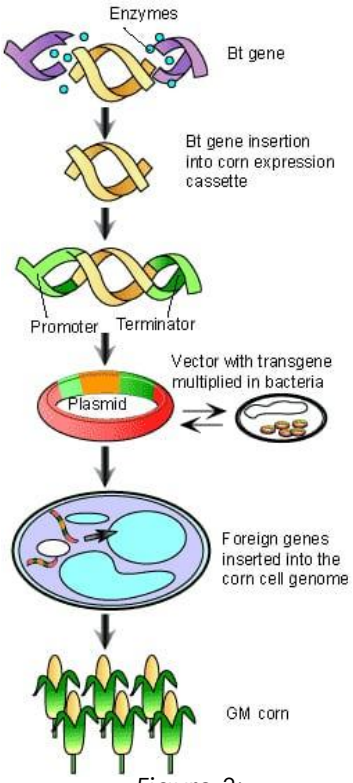

Figure 3: Engineering

On the right you can see a visual representation of what is done to get the Bt gene into the corn's DNA. We want to divide the entire process into four major steps starting with the DNA extraction from *Bacillus thuringiensis*. With gene extraction you can only extract the entire DNA at once containing your gene of interest, but also all the other genes of the organism. With that we get to the second step, which is the extraction of the gene of interest out of the bacteria's DNA. So you have to cut out your piece of interest from the DNA strand and that's possible with restriction enzymes. Restriction enzymes are able to locate a specific DNA sequence and break the strand right there.
Now we get to the third step, which is modifying the gene, so it works properly in a different organism. The most important change to make is to add a promoter sequence so the protein is expressed in certain parts of the plant in higher concentrations and also only during particular periods of time. In case of the corn borer it would be important that a high concentration of the protein is present inside the plant's stalk, the habitat of the corn borer's caterpillar. After that the gene has to get cloned so multiple copies of the gene are present which is important for the next step. For the cloning process you have to paste the gene into a plasmid (circular DNA sequence outside of chromosomes that replicates as well) with the help of DNA ligase. And now the goal is to insert this plasmid into an organism, which can replicate this plasmid. And an often-used organism is E. coli, which through a heat shock takes up these plasmids. Then through reproduction of E. coli the plasmids get replicated as well until you have enough genetic material and extract them out of the E. coli again to proceed with the process.
The fourth and most important step is the insertion of the gene into the corn's DNA. Since such a plant has millions of cells you can't insert the gene into every single cell one by one. The solution for this problem is to find a well-suited cell, which in this case is a totipotent cell. The benefit of this cell is that just a single can develop into an entire plant. So now you only have to insert the gene into this one cell. That can be done through multiple methods including the gene gun, agrobacterium, microfibers and electroporation. The goal of all these methods is to deliver the gene into the cell's nucleus without killing the cell. We wanted to take a closer look at two of the methods starting with the gene gun. The gene gun is used for genetically modifying plants since 1980. Microscopic metal particles of gold or tungsten get liberally coated with the gene and then fired into the cell with the hope that at least one gene gets picked up in the nucleus.
The second method we wanted to look at works with the help of an agrobacterium. This method makes use of *Agrobacterium tumefaciens*, a soil bacterium that works as a natural genetic engineer. These bacteria have an extra piece of DNA, which leaves the organism once it is inside a plant, inserting itself into the cell's DNA and resulting in the bacteria taking over the control of the cell.

Scientists took advantage from that and replaced the bacteria's piece of DNA with the gene of interest, in our case the Bt gene.
Once finally inside the nucleus of a totipotent cell, the Bt gene is present in every cell in the growing plant. These plants grow to maturity in greenhouses and the seeds they produce get collected and sold. With this entire process the Bt gene will be present in every cell of a corn plant growing from such seeds.

But what happens if a caterpillar now feeds on Bt-corn? The principle is actually pretty simple. The Bt proteins form bonds with specific receptors on the gut wall of the organism but not all animals show these receptors (for example all mammals do not have such receptors). If then too many of those bonds are formed pores in the gut wall will eventually form which finally leads to an uncontrollable passing of bacteria and ions through the gut wall. That's very damaging for the pest and it will eventually die.

4. Interview:

We also had some open question and we wanted an opinion of an expert so we looked for an interview partner in addition to our research. We found an expert from "Agroscope" in Zurich who was kind enough to answer our questions. His name is Michael Meissle and he has been working with Bt-corn since 15 years.

1. In how far is Bt-corn superior/inferior to conventional corn?

BT-corn produces one or more proteins from the donor bacterium *Bacillus thuringiensis*. These so called cry-proteins have insecticidal traits that are toxic for specific insects. Some are toxic for moths and butterflies and others are toxic for beetles. Some caterpillars, especially the corn borer, are very detrimental for the corn and cannot be fought with conventional insecticides because they live in the corn stalk. But the Bt-corn produces the cry-proteins all over the plant so all pests get contaminated with the toxin.
So Bt-corn is superior to the conventional corn because it produces the cry-proteins, which kill the pests. With that there are no more insecticides needed and after all a safer yield is achieved.
The downsides of Bt-corn are the requirements that follow the cultivation. Provided that it even is permitted, which isn't the case in Europe (except in Spain and Portugal). So the farmer has to make sure that no conventional corn is nearby the Bt-corn to prevent the fertilization of the conventional corn with Bt pollen. However the Bt-corn farmers also have to plant conventional corn within their Bt cornfield to prevent resistances. The idea behind this is that if a corn borer becomes resistant it fertilizes a non-resistant corn borer of the conventional corn so the offspring could end up to be non-resistant again.
And if the Bt-corn then doesn't stay on the farm the farmer has to label everything with "GVO". But these instructions and with them the effort is different in other countries.
In conclusion Bt-corn leads to a safer yield but then again the cultivation is connected to more effort.

2. What is your opinion on the medical problems, which occur with the usage of Bt-corn? What are those problems?

The Bt proteins harm the pest by bonding themselves to specific receptors in the gut of the targeted organism. Pores form in the gut wall where ions can travel through and damage the organism until it dies. If this receptor is missing there won't be harmful affects. Mammals (including humans) miss out on this receptor, which means that it's not harmful for us. The protein just gets digested like all the other proteins. Even though there were pieces of Bt proteins being found in human tissues it is not clear if they're harmful. In the US animals are being fed with Bt-corn since 20 years without problems. So the corn is being used to feed the animals which means that the humans are not in direct contact with the protein. The only exception is the sweet corn, which is directly consumed by humans. And of course all products for human nutrition must pass several tests before they can be sold. Until now there are no problems being discovered from these studies.

3. Does Bt-corn lead to a significant economic advantage?

People wouldn't use Bt-corn if there weren't advantages. The gene-manipulated seeds are more expensive than the conventional ones but in return the risk of a harvest failure through the target pests is lower and you don't have to spend as much money on pesticides. But of course it also depends on the region because if the problem with pests is big the advantage is larger than in regions where the pests aren't a big problem.

4. In how far is Bt-corn more efficient in cultivation and maintenance?

As described, if you use Bt-corn you don't have to use pesticides against the corn borer or other harmful moths. In some regions (USA but also Europe) the corn rootworm, a beetle, is also a big problem but Bt-corn is able to defend itself as against the moths. So that's the advantage in terms of maintenance but in terms of cultivation there's not really a difference.

5. Do large fields full of Bt-corn have an impact on the environment?

The fact that Bt proteins operate on very specific groups leads to very little problems with other organisms. There are hundreds of studies that support that. But there are discussions about possible affects on precious butterflies that shouldn't be attacked. The Bt-corn's pollen could end up on the feeding plants of the caterpillars. Modern Bt-corn sorts only show a little concentration of cry-proteins so that the butterflies are not badly affected. And also the beetle-specific toxins don't attack the untargeted beetles.
Harmful affects on soil organisms are not known even after years of Bt-corn cultivation.

6. We have read that the cultivation of Bt-corn in Switzerland is forbidden. But for example in the US the majority of the corn is from gene manipulated seeds. What is the reason for that?

In Switzerland we have the Gentech-Moratorium. The population voted for that so research could first be done to look out for possible problems before farmers are be able to cultivate Bt-corn. This moratorium gets prolonged more and more.
Currently there really isn't the need for Bt-corn in Switzerland because here we don't have big problems with corn borers.
Another reason against the usage of Bt-corn is the unpopularity among the people. With that the producers are scared to harm their image if they switch to gene-manipulated products.

7. Is it a goal for you and others to optimize Bt-corn or is it the goal to get rid of it and look for better alternatives?

Still a big problem is the development of resistances. At some places the farmers don't play along the rules when it comes to the concept of the refuges. And on the other hand the concept sometimes isn't really working because the concentration of the cry proteins may not be high enough and some of pests survive and develop a resistance. One method to prevent that from happening is the combination of multiple cry proteins in one plant. Right now the maximum of combinations is the so-called SmartStax corn with six different Bt proteins in it.
Currently researchers try to find proteins from other bacteria that may be better suited. Another goal is to find a mechanism that works in combination with Bt proteins.
And apart from that there has lately been a success with corn containing an RNAi-mechanism instead of toxic proteins. With that method some important genes in the pest can't get expressed anymore which finally leads to its death.

5. Discussion:

The progress made through the application of Bt-corn, especially in certain areas where pests could destroy the majority of the yield, is crucial. As our expert stated, Switzerland does not have to be as cautious about pests like the corn borer as our neighbors overseas, the USA and Canada. Therefore the application of said *(Bacillus thuringiensis)* is severely important for the food supply. Due to its built in defense mechanism against foreign intruders, it suppresses the populations of certain pests, which is obviously beneficial for the corn itself but also for surrounding crops in this area. What also comes with the genetically modified corn is that the use of sprayed insecticides drastically decreased since the initial release for large-scale production. This means, that the agriculture business saved a huge amount of money. But are there any risks and downsides to it? According to the science, there is no proof of genetically modified crops or BT-corn in particular harming consumers in any ways. As our interview partner also clarified, the receptors, which the proteins from the BT-corn bond themselves to, are missing in humans and mammals in general. Therefore, the toxins only harm the species, they are supposed to: pests. In addition, BT-corn seems to be no or at most a very small

threat to the environment surrounding the crop fields because of its specification, which is another crucial upside. But what farmers and scientists have to keep in mind are possible resistances that pests can develop. As described from our expert, if farmers don't obey to the essential rules, it can lead eventually to resistances. And it is the task of the scientist to minimalize the risk of said resistances. The consequence of resistant pests is the destruction of the product or in other words: the whole idea behind the BT-corn would be pointless.
Scientists and researchers are nonetheless pretty satisfied with the whole idea of taking advantage of an organism's proteins to fight enemies in agricultural production as for example the corn borer, the enemy of corn, is fought with the Bt protein. For the future in this sector scientists are on the hunt to find new bacteria with their specific genes that can help us fighting the pests. An expert, quoted in our introduction: "We're just scratching the surface and we're trying to better understand how they work since there is a diverse group of bacteria".
In addition, as our expert in the interview told us, there are also other methods that don't involve toxins in form of proteins that could be the future of fighting pests but we didn't go into detail with such methods because we wanted to set our focus on Bt-corn.

As consumers play an important if not the most vital role in this economic branch, it is up to the producers to keep the people happy with their product. As we learned from various news articles, the situation with GMO's is quite tense in Canada and the USA. We mentioned in the "Introduction" that the producers are intransparent about whether their product is genetically modified in some way or not. And the big corporations intend to keep the current situation as such. We and also the people of mentioned countries do not approve of that and are quite unsatisfied. We as consumers have a right to know exactly of what the products we buy consist. Even if they are no considerable health issues with genetically modified foods, it must be properly labeled in the recent future.

6. Summary:

To keep up the food supply is a big challenge for human kind. For example in the agricultural sector scientists in the 90's have started to genetically modify their crops in labs so the highest yield could get achieved, without having to spray insecticides. In this paper we, Jonas Blatter and Paul Mamot, wanted to take a closer look at one of these methods and to be more precise, the Bt-corn. We didn't understand the full practice behind this method and started to ask ourselves some questions: How does it even work? How does it protect the plant from pests? And also what are the downsides to these methods?
To answer these questions we did a lot of research and we also contacted an expert of "Agroscope", who could help us.
Here's a short rerun of the process: scientists take advantage of a bacterium, *Bacillus thuringiensis,* which produces a specific protein that is toxic for the pests of corn, one being the European corn borer, *Ostrinia nubilalis.* The first step in the process is the extraction of the bacterium's DNA. In a second step the gene of interest gets cut out of the DNA strand with the help of restriction enzymes. In a third step this gene has to get modified with a promoter that controls the presence of the protein in the different parts of the plants in various concentrations. With, for example, the corn borer being in the corn's stalk, the concentration there is therefore high. After that the gene gets cloned with the help of plasmids and *E. coli* bacteria. In a last step the gene has to get into the corn's DNA. To achieve that the gene gets shot into the nucleus of a totipotent cell with the help of a gene gun. This one cell (with the Bt gene successfully inserted) can then grow to maturity and the seeds it produces can get collected. The protein this gene produces is able to bond to receptors in the pest's gut. After a while pores are formed in the gut wall where an uncontrollable ion- and bacteria flow runs through, causing the pest to eventually die. Since these receptors aren't present in the gut of humans and in general mammals it shouldn't be harming for us as consumers. We couldn't find a lot of crucial downsides but one is the possible formation of resistances. Another one is that people aren't informed correctly as in how far the food is genetically modified. There's a lack of communication between the producer and the consumer. But otherwise we couldn't really find more downsides but maybe some reveal themselves in the future as research of possible problems is going on.
For the future scientists are on one hand looking for other bacteria that have helpful genes against pests and on the other hand they are trying to find a method that doesn't involve toxins in form of proteins.

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