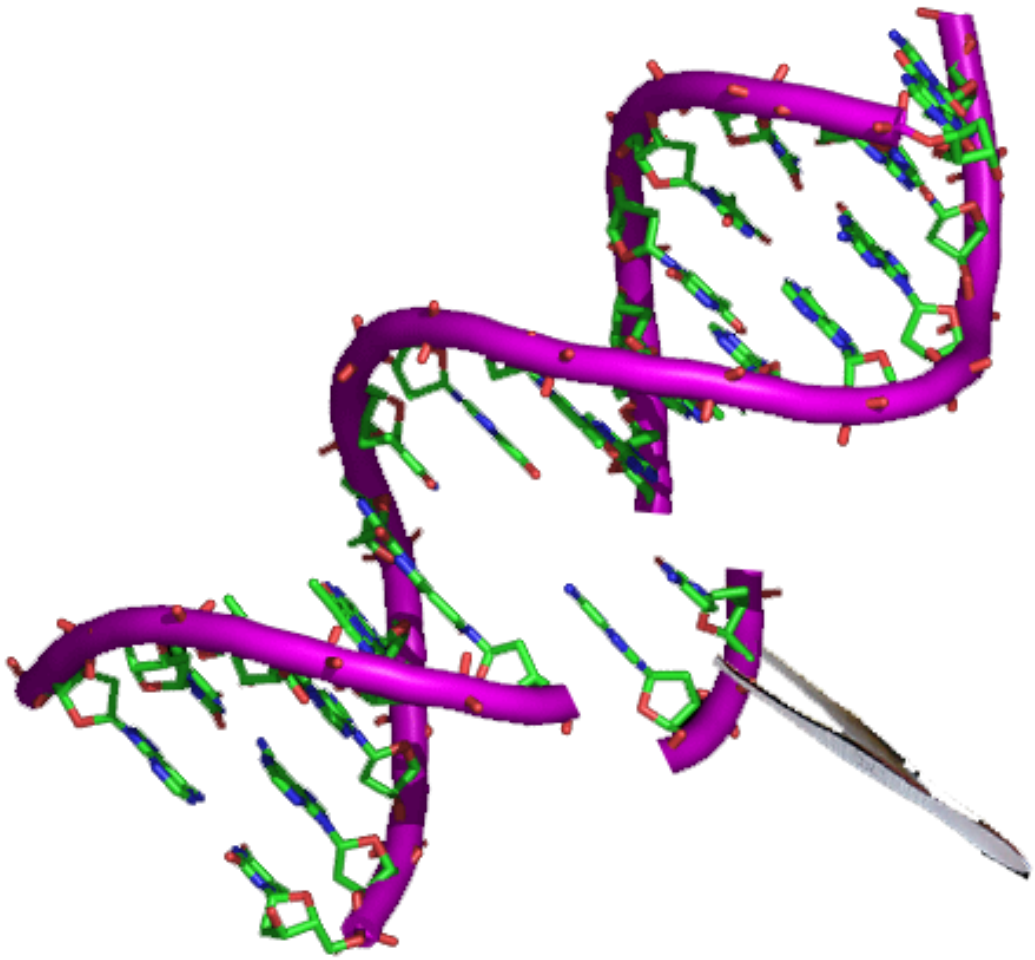
Gene Manipulation and Editing in Agriculture: The Long-Term Effects



L. Gebhardt, E. M. Sherringham

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Introductory note

We decided to write about GMO crops and their effect on the agriculture industry in order to get a clearer picture of how our future will be shaped by them. Supplying our society with food is one of the most important, if not the most important, tasks we humans have. Often, in our modern society, the average person will not be confronted with the fact of how, where and by whom his or her food was produced: and yet agricultural innovations and set-backs have the power to fundamentally change society and have, in the past, even led to various evolutionary changes in man's biology. Currently, genetic engineering seems to be the most discussed topic when it comes to agriculture and the bacillus thuringiensis gene among the most popular tools with which genetic engineers operate; recent discoveries in the world of gene editing (for agricultural purposes) (i.e. CRISPR-Cas9) finally convinced us of the importance and relevance of covering the specific topics we now present in this paper.

1. Introduction

In this paper we will examine the impact of genetically modified crops in agriculture as well as the future of genetic engineering in agriculture. The purely technical aspect will cover bacillus thuringiensis and its use as an insecticide (both as an 'organic' insecticide and as the donor of a gene that is then artificially implanted into a plant (genetic modification) and then venture into a relatively new in territory in biology with CRISPR-Cas9, a tool bound to define the future of genetic engineering. Then the consequences and benefits, the pros and cons of the technologies described will be explored. Finally, an interview with Cesare Gessler addresses both ethical and practical issues concerning innovation in the field of genetic engineering.

2. Technical Part

1. Build

Bacillus thuringiensis is a commonplace bacterium (FAS, 2011) found in 'soil, water, dead insects, dust from silos, leaves from deciduous trees, diverse conifers, and insectivorous mammals, as well as [in] human tissues with severe necrosis' (Palma, Munoz, Berry, Murillo and Caballero, 2014). Different variants kill a diverse array of insects and other invertebrates (Mahr, 2016). As is the case with most bacteria, due to a lack of resources, bacillus thuringiensis can undergo a phase of 'sporulation' where a firm outer shell forms to protect DNA from various external conditions, rendering it almost dormant but very persistent (World of Microbiology and Immunology, 2003).

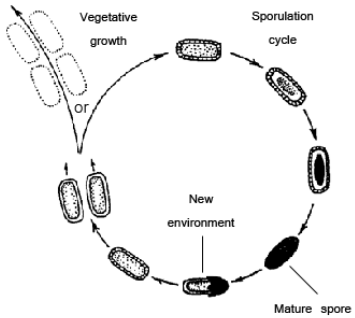


Image 1: The basic life cycle of bacteria. During the vegetative growth phase, bacteria reproduce via a process called binary fusion (Sahathanee Group, 2008).

During sporulation *bacillus thuringiensis*, or BT as it is commonly called, synthesises Cry (crystal) and Cyt (cytolytic) proteins as 'crystalline inclusions' around its spore: these lend it its insecticidal properties (Palma, Munoz, Berry, Murillo and Caballero, 2014). Cry and Cyt are a *family* of toxins, meaning there are many sub-types of them in existence (Pigott, King and Ellar, 2008). There are thousands of different kinds of BT: together they produce 200 different varieties of proteins toxic to specific insects (Mahr, 2016).

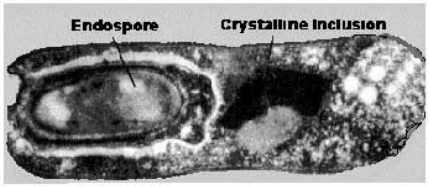


Image 2: Sporulating BT cell

BT can also synthesise toxins during the vegetative phase (the growth phase), releasing so-called VIPs ('vegetative insecticidal proteins') into its nearby environment (Palma, Munoz, Berry, Murillo and Caballero, 2014). Discovered in the mid-1990s, VIPs are distinct from Cry and Cyt proteins and do not target the same insects as the latter, although they do kill similarly (Watkins, Huesing, Margam, Murdock and Higgins, 2012).

2. Insect Death via BT

BT toxins mostly target insect larvae: lepidopteran larvae (caterpillars), for example, eat the leaves and stalks of plants while coleoptera larvae (beetle grubs) live off the roots of plants (Hellmich, 2012). Left to their own devices, such pests cost farmers billions of dollars annually (Hellmich, 2012).
The Cry toxins in bacillus thuringiensis are not harmful to humans, whose guts are acidic: in order to release the toxin from the crystal, an alkaline gut is required; environments with high pH values dissolve the otherwise insoluble crystal (Sanahuja, Banakar, Twyman, Capell and Christou, 2011). Once solubilised, proteases in the midgut cut/cleave the protoxins, activating them (and turning them into *toxins*) (Sanahuja, Banakar, Twyman, Capell and Christou, 2011). The active toxins then attach to gut membrane receptors; at this point, the insect stops eating (Arifeen, 2014). A few hours later (Arifeen, 2014), the pores open, the membrane now destroyed: this allows BT spores, as well as the contents of the gut to enter the bloodstream, leaving the insect to die of septicaemia within 1-2 days (Bessin, n. d.) (Vinje, n. d.) (Arifeen, 2014).

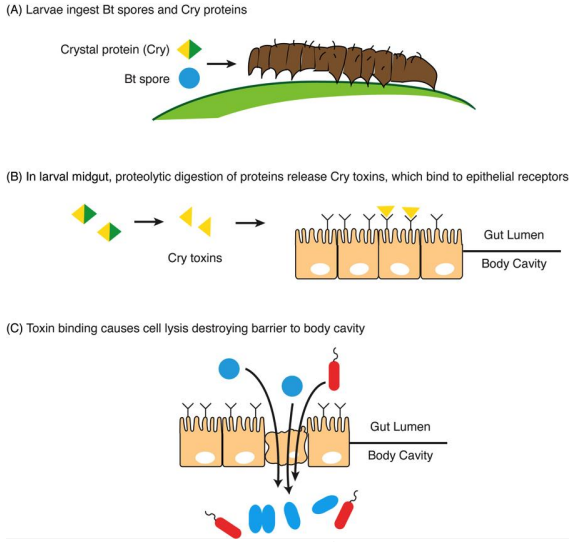


Image 3: Insect death caused by ingestion of BT

3. Non-GMO uses/History

In 1901, biologist Shigetane Ishiwatari identified *bacillus thuringiensis* as having caused the large-scale decimation of silkworms, making him the first to isolate BT as an insecticide (UCSD, n. d.). BT got its name in 1911, when it was (re-) discovered in Europe by Ernst Berliner, who named it after the place where he had found it, Thuringia (UCSD, n. d.). BT began to be used as an organic pesticide in the 1920s and was commercialised by the 1930s (UCSD, n. d.): it was

sold as a spray and made in large fermentation tanks (Mahr, 2016). Seeing as there were relatively few subtypes of the bacterium known at the time, it wasn't widely applicable (UCSD, n. d.). Unlike synthetic insecticides, it would not kill a large variety of different pests at the same time (UCSD, n. d.). Crucially, BT insecticide sprays were unable to target underground and stalk-boring pests (UCSD, n. d.). For a long time, these and other factors, such as BT being easily washed away by rain and disintegrated by the sun's UV rays (FAS, 2011), made it an unpopular choice for farmers. In the 1980s, however, insects had become increasingly resistant to synthetic insecticides: BT sprays were now regarded the organic alternative, seeing as they did not persist in the environment for very long after they were used and didn't wipe out each and every type of insect they came in contact with (UCSD, n. d.). In the mid-1980s, scientists experimented with inserting the BT gene responsible for emitting toxic proteins into tobacco, so that it would be expressed in the plant tissue (Sanahuja, Banakar, Twyman, Capell and Christou, 2011). Although this transgenic tobacco was never commercialised, trials with BT potatoes, cotton, corn and rice proved of interest commercially (Sanahuja, Banakar, Twyman, Capell and Christou, 2011). The first genetically modified BT products hit the market around 1995 (Sanahuja, Banakar, Twyman, Capell and Christou, 2011).

4. The Making of BT Crops

In order to create a transgenic plant, a foreign gene (coding for a specific trait) has to somehow be inserted into the DNA of a selected plant. How can this be achieved? In genetic engineering, tissue samples of plants are used to grow a large number of homogenous cells which serve as the 'plant' being artificially introduced to the new gene (Hain and Ehly, 2000). In the case of BT crops, the new gene being introduced is the one that produces Cry toxins: this gene has to first be isolated (Romero, 2008). A sort of 'copy paste' mechanism is used for this purpose: restriction enzymes attach to specific nucleotide sequences, cutting the DNA strand in question (CSH, n. d.). They also sever circular plasmids (plasmids are found in bacteria and are made up of DNA: they reproduce independently of regular chromosomal DNA) (Scitable, 2014): another enzyme, ligase, then fuses the plasmid and Cry DNA strands together (CK-12 Foundation, n. d.).

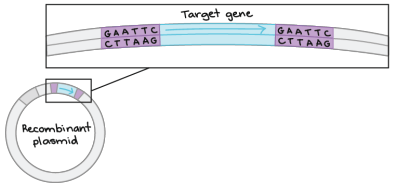


Image 4: Result of the process described above

This new DNA combination is then multiplied to ensure a practical amount of the gene to work with later on (Romero, 2008). Following additions to the recombined DNA strand are also necessary: a promoter sequence to determine where in the plant and at what time the gene will be expressed (it functions as a sort of on/off switch (Romero, 2008)), a termination sequence to mark the end of the DNA strand and marker gene that expresses an obvious trait so that it is clear to researches which cells/plants have the transgene (Bessin, R., n. d.). Proteins that code for antibiotic resistance are often used as markers (Romero, 2008).

Image 5: The newly designed gene

This gene must now find its way into the nucleus of a plant cell, reaching the chromosomes so it can become part of the plant's DNA (Hain and Ehly, 2000). One way to achieve this is by using a 'gene gun', also called 'particle acceleration or microprojectile bombardment' (Hain and Ehly, 2000). A gene gun is made up of two compartments: a vacuum chamber where the bombardment essentially takes place and a pressure chamber where helium builds up pressure (Taylor, 2013). This pressure is needed to create a shock wave that breaks the so-called 'rupture disk' (made out of plastic) which in turn hurtles an enclosed DNA-coated nanoparticle (either gold (Hain and Ehly, 2000) or tungsten) straight towards the nucleus of a plant cell. The DNA (the Cry gene, in this case) then detaches from the nanoparticle, inserting itself into a plant DNA strand without eliminating any of its already existing DNA, its existing genes.

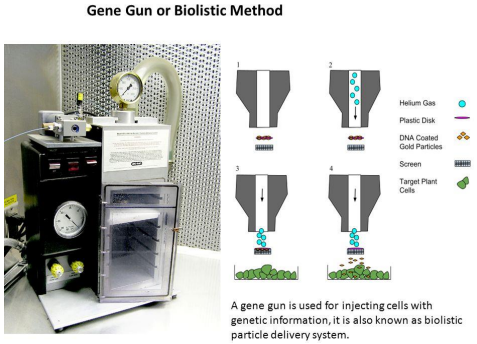


Image 6: Gene gun and bombardment process

5. CRISPR-Cas9

Instead of modifying genes, i.e. artificially inserting foreign DNA into already existing DNA, the CRISPR-Cas9 complex simply edits existing genes (Li, 2018). It originated in bacteria combating viruses: the Cas9 enzyme would cut identified virus DNA, thus hindering a cell take-over (Molteni, 2017). The so-called CRISPR ('Clustered Regularly Interspaced Palindromic Repeats') evolved in order to better identify strands of virus DNA: Cas9 stores some of the virus DNA (now a so-called 'spacer' (Synthego, n. d.)), making it easier for the enzyme to recognise which strands to cut in the future (Molteni, 2017). This vital information is spaced out between repetitive, meaningless genetic information (Max-Planck-Gesellschaft, n. d.) (Molteni, 2017). Together both kinds of DNA form what is called a 'CRISPR array' (NLM, 2019). The CRISPR array is then transcribed into CRISPR RNA (crRNA) (Synthego, n. d.). After this, base pairing with another form of RNA, called trans-acting or tracrRNA, takes place; the Cas9 attaches to

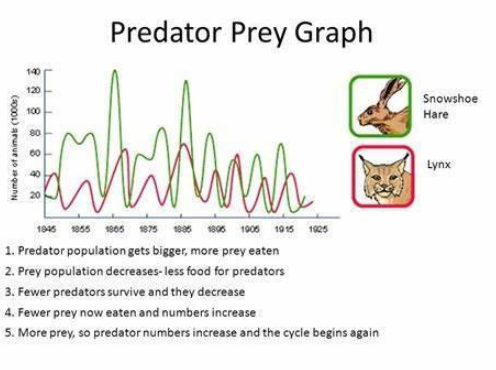
this tracrRNA (Synthego, n. d.). One part of the resulting ‘guide RNA’ forms a loop to ensure it remains attached to the Cas9 enzyme while the other part extends outwards, ready to interact with potential viral DNA (Molteni, 2017). However, when the guide RNA comes in contact with viral DNA, how come it doesn’t cut itself, seeing as it has seemingly identical viral genetic information? Next to the shared genetic code information in the foreign DNA (also called the ‘protospacer’) there exists what is called the PAM (Protospacer Adjacent Motif), a sequence of about 2-6 nucleotides (Synthego, n. d.). The guide RNA does not have this sequence of nucleotides incorporated into its own genetic information (Synthego, n. d.). The Cas9 enzyme will not latch onto or cut the viral DNA without there being a PAM (Synthego, n. d.). Once Cas9 has attached to the PAM, the guide RNA begins to unwind a section of the viral DNA’s double helix, one strand forming base pairs with the matching guide RNA (Nature, 2017). Cas9’s two nuclease domains then cut both strands of the DNA: in the process of the cell unsuccessfully trying to reattach the parts, mutations will often occur, leading to the complete destruction of (a) certain gene(s) on the DNA strand (Nature, 2017).
Instead of combating viruses, scientists use the CRISPR-Cas9 complex to edit genes responsible for diseases or subtracting from crop yield (Li, 2018). In the lab, once they have discovered/selected DNA they want to eliminate, researchers engineer RNA to correspond to and target specific genes (Molteni, 2017). Once the gene is eliminated, alternative DNA sequences can be implanted, repairing the gene (Molteni, 2017). Overall, working with CRISPR-Cas9 ensures a faster and more precise process compared to other forms of genetic engineering (Li, 2018).

3. Discussion

The reason why big companies like Monsanto (now Bayer) or Syngenta (now Chemchina) want to produce genetically modified plants (mostly corn, wheat, soy and cotton) is because they say that by using their genetically modified seeds, farmers would have to use less pesticides and could earn more from areas under cultivation. On Monsanto’s website they say: “We exist to serve famers.” (https://monsanto.com/innovations/ (14.03.19)); they claim to have found a means to increase yields and still be sustainable and protect the environment. They say that genetically modified food is just as nutritious and safe as non-GMO (‘genetically modified organisms’) food. By using their more efficient products, farmers could fight world hunger (in itself a legitimate goal). It *is* true that due to the modern pesticides and GMO crops, farmers are able to cultivate much bigger areas with less people. During the first few years, they can achieve enormous yields from a (equally enormous) field of one specific crop. Giant amounts of food are thus produced. Monsanto lists studies that seem to support their position in every regard.
Adversaries of this method counter by saying that these studies do not sufficiently prove—due either to the time period of their observation or their scale—the safety of GMO crops for the environment, animals and humans. Furthermore, they say that there isn’t enough money for independent studies to be conducted because big biotechnological concerns play a very important role in funding research.

They point out, for example, that by using Bt corn, the pressure on the pest increases massively, which leads to a faster formation of resistances because only those that develop a resistance survive (like the link between prey and predator, see image 1 (p. 9)). That “using only a single tool […] can encourage species of weeds to develop resistance to that tool,” (https://monsanto.com/innovations/crop-protection/ (14.03.19) is a fact on which Monsanto agrees: it is exactly for this reason that the they themselves should use a combination of approaches, which normally includes another pesticide. Farmers end up having to use more pesticides than before in order to protect their crops: with Bt corn, for example, its constantly released toxins accumulate with the other treatments that have to be used (often a herbicide and another insecticide) should the pest develop a resistance against the toxin released by the Bt corn.

Image 1: The relationship between predator and prey



This scenario is true for the cotton worm, which has become resistant against the toxins released by Bt cotton. The farmers who thought that the more expensive seeds were a good investment must now buy more (equally expensive) products, produced by the same companies which produced the seeds. In some cases, farmers say that they cannot even defray their original costs.

Monsanto, however, says that Bt corn and Bt cotton have helped farmers bring the corn rootworm and the cotton bollworm under control. (https://monsanto.com/innovations/research-development/crop-protection-listen-innovate-repeat/(14.03.19))

Image 2: The corn rootworm

Another issue for GMO adversaries is the patents that the seed producers hold. They say that it is not right for a company to own a living creature that reproduces itself and changers. Holding a patent of a living creature, in this case a plant with a specific gene, means the patent holder owns every plant which has that gene and all farmers have to buy new seeds every year; to grow them on their own is now, after a long history of breeding, illegal.

This leads us to the next problem they see: the contamination of non-GMO plants. Once released into nature, there is no controlling the spreading of these plants. It is possible that through flying pollen, non-GMO plants can get contaminated with the new gene, even though the owner didn't do anything to obtain it. The patent holders of the plants can then accuse them of infringing their patent. This happened to Percy Schmeiser, but after appealing to the Canadian Supreme Court, the court held that he did not enjoy any of benefits of the GMO crops and that he thus owed Monsanto nothing (https://www.agpolicy.org/weekpdf/202.pdf (15.03.19).

Moreover, with old, traditional varieties getting supplanted (the same goes for insects), the contamination of non-GMO plants could result in decreased biodiversity. The preservation of these varieties is crucial because the different sorts have all adapted to specific conditions and pests and may be the key to new sorts with better pest resistance. If there are only four varieties of a plant and they all lack said resistance, all of them will be infested. Having a small range of varieties makes our ecosystem more vulnerable for all sorts of pests or diseases.

The lack of declaration laws concerning GMO crops in the USA is another problem, seeing as it prevents customers from choosing freely what food they want to eat and which method they want to support. Because the food is not labelled, big studies on humans' reactions to GMO crops are near impossible. In 2016, however, the US government passed a law on how to label GMO-derived ingredients in food.

What scares adversaries of GMO crops further is that big biotechnological companies can gain more and more power over all seeds and food in general by merging with the other big concerns, like Monsanto and Bayer did on March 21st, 2018.

It is difficult to tell which scientists and studies are most trustworthy because the topic has become a very emotional one, where both sides are very one-sided and extreme in their results and opinions. Nevertheless, the arguments of GMO adversaries are more convincing, for if concerns like Monsanto really "exist[ed] to serve the farmers" (), they would not claim patents that inhibit farmers from breeding independently. If the corn rootworm and the cotton boll worm were under control, there wouldn't be so many farmers telling a different story. Furthermore, the fear of contamination seems to be justified. It should not be forgotten that the trade with seeds and pesticides is a huge business.

However, not all GMO crops need be considered dangerous. There are events where the method of gene manipulation is used to accelerate only the breeding process, where no gene which is foreign to the species is incorporated in the genome. With the CRISPER-Cas9-method it is even possible to incorporate a cognate gene with higher resistance to a disease without having to use markers like antibiotics, which would again increase the pressure of selection for resistant bacteria. Scientist Cesare Gessler, for example, takes the resistance gene of a wild type of an apple and incorporates it into the genome of a new type of apple, which lacks the resistance to apple scab. His goal with this method is to provide an alternative to the use of pesticides, which is no different from the natural breeding process. Additionally, since the natural breeding process is only accelerated, not altered, he claims that this method should be free to use in biological cultivation too (https://www.youtube.com/watch?v=ZXb-MbKxICM (16.03.19)). If he is right and including a new but cognate gene into the genome of a plant does not alter the natural breeding process, it seems as if this could be a way of using gene manipulation which is not dangerous, but sustainable.

4. Interview with Cesare Gessler (In German)

Sie haben davon gesprochen, dass das Einbringen von artgleichen Genen in eine Pflanze kein neues Risiko darstellt, da es einem verkürzten, natürlichen Züchtungsprozess entspricht. Ab wann unterscheiden sich Gene so stark von denen des Untersuchungsobjekts, dass die Folgen, die das Untersuchungsobjekt auf die Umwelt haben kann, nicht mehr eingeschätzt werden können? Und ab wann würden Sie gewisse Folgen als gefährlich einstufen? Umfasst das «sichere» Spektrum nur dieselbe Art, oder mehr?

Grundsätzlich sind artgleiche Gene (oder alle Gene die natürlich einkreuzbar sind) schon «getested», was nicht bekannt ist, ist die Auswirkung des unnatürlichen Einbauorts. Wobei die Wahrscheinlichkeit wohl gegeben ist, dass dieser zu unerwünschten Effekte führen könnte, wie schlechte, kein Wachstum oder genereller negative Auswirkungen. Dass hingegen Superpflanzen, neue toxische Substanzen entstehen ist unwahrscheinlich und auch nicht plausibel erklärbar.

Artfremde Gene nach meiner Ansicht sind nicht das eigentliche Problem, sondern welche Gene, respektive neue Funktionen die wir einbauen. Zum Beispiel Gene für Resistenz gegen ein Herbizid (Glyphosat-Monsanto) sind kein Problem allein gesehen. Das Problem ist Glyphosat (vermutlich kanzerogen) und die Verwendung von Glyphosat-Samen als Paketverkauf, mit der Patentierung. Also auch die ganze Abfolge der Monokulturen, Vereinfachung der Kultur, usw. Als zweites Beispiel: Einbau (Unterdrückung) der Wachstumsregolatoren im Lachs der damit zu grösserem Profit, aber auch zu einem potenziellen Superpredator führt, somit sehr gefährlich falls er in die frei Natur entweicht.

Klar, es ist immer der Gesichtspunkt. Für mich ist der Einbau von Resistenzgene gegen Krankheiten immer positiv, für die Farma hingegen negativ da weniger Pestizide verkauft werden.

Wenn bei einem genmanipulierten Organismus nach der Manipulation nicht mehr festgestellt werden kann, dass er genetisch verändert wurde, ist es dann legitim diesen patentieren zu lassen? Und wenn ja, wie soll das Auskreuzen verhindert und überprüft werden, ob ein Organismus durch Züchtung oder genetische Manipulation entstanden ist?

Meine sehr persönliche Ansicht: keine Patente auf DANN: Die Forschung und Entwicklung soll von der Öffentlichkeit gefördert werden und Allen zugutekommen (Umwelt!!). Bei Genen die auch natürlich eingekreuzt werden können, ist das Auskreuzen kein Problem, feststellbar ob ausgekreuzt vom original Spender (z.B. Wildapfel) oder von einem GMO (Gen und eventuelle Promotor /Terminator am richtigen Ort) von schwierig bis unmöglich. Man könnte eine Erkennungssequenz miteinbauen.

Ist es sinnvoll mit aufwendigen Methoden resistente Apfelsorten zu entwickeln, wenn man Landwirten damit ermöglicht weiterhin in Monokulturen anzubauen? Wäre es nicht wichtiger ein Verfahren zu entwickeln, mit dem Äpfel in Kombination mit anderen Pflanzen und nachhaltig in Bezug auf Nährstoffverbrauch und Krankheitsbefall angebaut werden können?

Diese Frage ist nicht zu beantworten: »leider» sind Monokulturen aus ökonomischen Gründen gegeben.

Sinnvoll hingegen sind resistente Apfelsorten (gezüchtet oder GMO), sehr sinnvoll da sie eine Reduktion bis Verzicht von Pestiziden ermöglichen. Einsparung für den Produzenten, Natur (Wasser Flüsse) nicht kontaminiert, weniger/keine Rückstände auf und im Produkt.

Gibt es noch einen weiteren Nutzen Ihrer Forschungsergebnisse, neben der Tatsache, dass es möglich ist Äpfel gegen weitverbreitete Krankheiten resistent zu machen?

Nein, das genügt.

Wie lange wird es Ihrer Meinung nach dauern, bis Ihr Verfahren grossflächig kommerzielle Anwendung finden wird?

Keine Ahnung, sicher nicht solange Pestizide, billig erhältlich sind und gewisse Organisationen durch die Verbreitung von Angst (Fake news) Geld sammeln können.

Tragen Wissenschaftler allgemein die Verantwortung dafür, was sie durch ihre Forschung ermöglichen?

Ja, absolut bei einer Anwendugsorientierter Forschung, bei Grudlagenforschung schwierig zu beantworten. Z:B Gentransfer kann negativ sein (siehe oben) oder sehr positiv, es ist die Anwendugsorientierte Forschung die den Ausschlag gibt.

Wie erhoffen Sie sich die weitere Verwendung Ihrer Forschungsergebnisse?

Reduktion des Pestizid-Einsatzs. Klare politische Entscheidungen basierend auf wissenschaftliche Kenntnisse (nicht Emotionen und Schlagwörter) im Interesse der Allgemeinheit, (was für GMO-Produkte wollen wir, welche sind von Nutzen für Umwelt und Gesellschaft).

Wie schätzen Sie die Einstellung der Bio- Landwirte und die der Konsumenten von Bio-Produkten ein? Werden sie tendetiell aufgeschlossener gegenüber solchen neuen Methoden oder lehnen sie sie immer absoluter ab?

Bio ist eine Statusquo und kann demzufolge nicht evolvieren. Es wäre wünschenswert, das Positive von Bio zu übernehmen, aber auch neue Konzepte, Produkte.

Nicht nur das, sondern auch neue (im letzten Jahrhundert nicht voraussehbar) Grundlagen zum Beispiel Berücksichtigung Co2 Output, Wasserverwendung, Energie.

5. Conclusion

We have come to the conclusion in this paper that the genetically modified crops big companies are selling to farmers and small businesses are not, to say the least, a good long-term investment and could lead to serious ecological problems future generations shouldn't be tasked to deal with. Technologies such as CRISPR-Cas9, which involve gene editing rather than gene modifying, however, appear to be less risky and even more efficient when it comes to crop innovation.

6. Final Thoughts

In writing this paper, we were introduced to the intricate nature of genetic engineering and to the macroscopic effects it can have on our environment and our society. It taught us the primacy of thinking ahead, of thinking long-term, as well as the importance of detailed analysis when it comes to biological innovation.

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Image 1:

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Images:

Image 1:

No author (n. d.). *The basic life cycle of bacteria.*

Available at: http://www.sahathaneegroup.com/articles/199940/55439

Image 2:

Bajwa, W. (n. d.). *Sporulating* Bacillus thuringiensis *cell.*

Available at: https://www.learner.org/courses/biology/archive/images/1022.html

Image 3:

No author (n. d.). *Insect death caused by ingestion of Bt.*

Available at: http://sitn.hms.harvard.edu/flash/2015/insecticidal-plants/

Image 4:

No author (n. d.). *Recombinant plasmid.*

Available at: https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/a/restriction-enzymes-dna-ligase

Image 5:

No author (n. d.). *The newly designed gene.*

Available at:
http://whatisbtcorn.pbworks.com/w/page/12449526/Biochemical%20Explaination%20of%20Bt%20Corn

Image 6:

No author (n. d.) *A gene gun and the bombardment process.*

Available at: https://slideplayer.com/slide/9414039/