# Keeping up with the plant killers

I gave this talk in 2020, the International Year of Plant Health, to discuss the pathogens that infect our crop plants. You can see that this talk dates to April 2020—so, while we were all thinking about a viral pandemic, other pandemics, pandemics of plant diseases, were threatening our crops. Here, I start with a brief illustration of the power of plant pathogens to re-shape plant development, then explore how other plant killers affect the crops we depend on for our survival and how plants protect themselves against these killers.

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**Rocky Mountain Pseudoflowers**

Alpine meadows north of Gothic. Colorado.

In happier times, we roamed the world on trips of biological discovery. Back in 2007, with my student Liliana Cano, who is now at the University of Florida, I travelled to Gothic in the middle of Colorado in the Rocky Mountains. If you go past this ancient, deserted mining town and up into the mountains, you'll end up in these beautiful alpine meadows at about 3000 meters of elevation or so. When you stroll through these meadows, you'll see these yellow "flowers". I would forgive you if you think they are flowers. Many students who go on collecting trips will confuse them with flowers.

Fungal mimicry of plants — "fake" flowers triggered by the rust Puccinia monoica on Boechera stricta.

But these are not flowers; these are plants infected with a fungus, *Puccinia monoica* as my colleague Bitty Roy discovered back in 1993. It's a rust fungus, and its host is a classical member of the Brassicaceae, *Boechera stricta*. An uninfected plant is shown on the right side, with its flowers, which are typical of the Brassicaceae. When it infects its host, the fungus completely reprograms the plant—you can see that the fungus has changed the development of the plant so that it produces erect leaves that are essentially mimicking a flower. These yellow 'flowers' even produce a scent. The fungus shuts down the production of the plant volatiles and makes its own, which are very attractive to

insects. The fungus thus turns the plant into a flower mimic to attract insects and promote its own reproduction. In fact, when scientists isolated the infected plants from insects, the fungus couldn't go on with its reproductive cycle—it really needs to attract insects to survive. To do so, it takes over the biology and development of this host plant and turns it into what we like to call a zombie plant, because this plant is not going to flower. It is "game over" as far as the plant goes.

Biological processes altered in pseudoflowers.

*Puccinia monoica* is not particularly important in agriculture or in crop systems, but I love this case because it's such a beautiful, vivid example of how pathogens can take over and manipulate their host plants. You can imagine all the modifications that are taking place at the molecular level when this infection happens—we have studied this a little and found that the fungus modifies dozens of plant processes. You can see that on the graph on the left side. All the plant processes that are modified by the fungus are shown in color (green is down, red is up). Anywhere from modifying plant hormonal balance to reshaping the plant developmental switches. It's a total take over. Other microbes also turn plants into zombies using some of the most amazing pieces of molecular trickery. We'll talk later about the work of my colleague and partner in crime Saskia Hogenhout at the John Innes Centre who figured out recently how a bacterium slows down aging in plants turning them into living dead zombies.

Other species of rust fungi threaten our crops and food supply. The most infamous are the ones that infect wheat, the most important source of food calories for humankind. These fungi are simply amazing, not just

e rust fungi also threaten our crops, such as wheat. And look at all the ons of spores they produce. I received the rust spore cloud image from ıde Wulff. The photo was taken in Australia but its origin is unclear. The rust spore covered boot is from a tweet by @WestBredWheat.

for the complex ways in which they colonise their hosts but also for the sheer number of spores they produce. This photograph was taken in a wheat field in Australia and you can see the orange cloud of spores behind the tractor. If you go on a stroll in these fields, then you'll be covered with spores. Like for the coronavirus we keep hearing about and the new mutants they spawn, the sheer population size of these rust pathogens makes them absolutely formidable evolving machines. Rust fungi can keep on producing new races and overcoming plant resistance. Imagine all the genetic variants that one single infected field would harbor.

### *Phytophthora infestans*, the "plant killer"

Potato blight caused by Phytophthora infestans.

It's not just the rust fungi that threaten our crops. All sorts of microbes and pests make a living infecting plants. Even some plants, so called parasitic plants, can infect other plants. I'd like to add a few words about potato blight, because this is what I worked on for most of my career. This is the aptly named *Phytophthora infestans*. *Phytophthora* is Greek for plant killer. The photo shows why. There is a resistant potato plant in the middle flanked by susceptible plants around it. It's very clear that when the potato is susceptible, it's completely wiped out by this pathogen. Of course, it's famous as being the Irish potato famine pathogen, which sparked so much misery back in the 19th century. It triggered those dramatic events that led to so much death and immigration. A little tidbit of information that always sticks in my mind is that the population of Ireland has never recovered to pre-famine levels. That just tells you about the impact that this pathogen has had.

The time-lapse video of Phytophthora infestans infection of potato was produced by Remco Stam. For more on the history and genomics of the strain of P. infestans that triggered the Irish famine, see Yoshida et al. eLife 2013. See also this PlantVillage blog post for general background on the oomycetes.

Here's a video that a former student, Remco Stam, now at the Technical University Munich, has produced. This is a time lapse of potatoes sprayed with spores of *Phytophthora infestans*. You just see that it's very sad. The music is very appropriate. In just a few days, the plants are completely destroyed. This is what *Phytophthora* does to potato—just a few spores will be able to penetrate the plant tissue, the leaf tissue, and colonize it very aggressively. The plants will be dead within a few days. In fact, this time lapse was done over four or five days maximum. The same could happen in a potato field.

/tophthora infestans (red) infecting plant tissue. This beautiful shot of the hemibiotroph Phytophthora infestans was taken by Sebastian Schornack.

If we zoom in on an infected leaf and use some molecular tricks—in this case, *Phytophthora infestans* is fluorescing in red because we have it express a fluorescent protein from jellyfish to visualize it—you'll see this network of hyphae that is growing into the plant tissue. Right at the edge, you can see that the pathogen is doing a very good job infecting very healthy, green plant tissue. Somehow, it's evading any detection from the plant and the plant is not able to detect that it's being invaded and fight off the pathogen.

One of the first questions that my lab is interested in is how do pathogens infect plants? There's a multitude of organisms, nematodes, bacteria, fungi, oomycetes like *Phytophthora*, viruses, aphids, insects, even parasitic plants, that can infect plants. Plants are continuously being attacked by other organisms that want to feed off them. But how do these organisms do it?

**Effectors: key to infection**

How do pathogens infect plants? They secrete effectors to modify host plants.

What we now know is that these pathogens and parasites are very good at modifying their host plants. They do so by secreting proteins, molecules we call effectors, into the host plant cells. A nice metaphor is to think about snakes and the venom they produce. When a snake bites a mouse, it's producing a venom that is, for example, paralyzing the mouse. That venom molecule that acts on the mouse is essentially a toxin. It's very similar in concept to what a pathogen does with its effectors. The pathogen secretes the effector inside the host plant to modify it in a way that's favourable for the microbe. The effectors modify host plant processes, and that's how pathogens colonize plants.

Therefore, effector genes in the pathogen have an 'extended phenotype. The definition for this goes back to a book by Richard Dawkins, *The Extended Phenotype*, which defines the phenomenon of extended phenotype as "parasite genes that have phenotypic expression in the host." This is a beautiful way to illustrate what an effector is. Basically, the gene of that effector is sitting in the microbial genome, but the protein itself is not functioning inside that microbial cell—rather, it is functioning in the plant cell.

A striking example here is taken from the work of Saskia Hogenhout whom I mentioned earlier. She works on phytoplasma; like the rust fungus I described above, these bacteria also make zombie plants. You can see that when the plant is infected (on the top right), there's no flowering anymore. Plant development is completely perturbed. Saskia

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:ctors — sensus Richard Dawkins in “The extended phenotype: the long ach of the gene”. The example of phytoplasma effector SAP54, which ransforms flower development, is from the lab of Saskia Hogenhout.

actually cracked what happens here, finding that the phytoplasma has an effector gene called *SAP54* that is responsible for making zombie plants. When she introduces SAP54 into a plant, then that effector switches off plant development in a way that turns that plant into a zombie plant that does not flower but keeps on producing green tissue. You can imagine that for the bacteria, this is fantastic, because it’s making that plant live longer, produce more green tissue, the bacteria reproduce more and is even more likely to attract an insect vector that would carry it to another plant.

Remarkably, these bacteria don’t have one such effector but a whole bunch of them. Another effector Saskia and her team studied recently uses a different molecular trick that slows down aging and makes the plant almost live forever. As the New York Times writer Veronique Greenwood described it, it’s a never-ending cycle of “Night of the Living Dead-meets-Dracula.” She described the parasite as turning plants into something between a vampire that never ages and a zombie host.

That’s the concept of effectors, a central concept in our field. We now know that all these pathogens, all these parasites, produce effector molecules. If you like, they are the plant pathogen venom.

**Effectors: key to immunity**

Now let’s switch to immunity. Saskia and I went for a walk a few days ago—I guess we’ll be doing more of that in lockdown too. But when we go for walks and look at plants, they’re surviving just fine. You will see an occasional diseased leaf here and there, but these plants are quite immune to most pathogens. And that leads me to another concept that is central to plant pathology, plant immunity. This concept dates back to the early days of plant pathology. Whenever someone identified a pathogen, they found out that most plants are resistant to that pathogen.

Now if you're an entomologist, you would phrase that differently. Entomologists would say, "Oh, most insects are specialists; they can only infect a few plants." But that's really the same thing. Another way to look at it is that most plants are able to defend against most pathogens (and insects). Disease is really the exception, not the rule. There's very few cases where a pathogen would be able to do the trick and find, for example, the right lock and have the right key to unlock the system of a plant to infect it.

Most plants are resistant because they have an immune system; just like any other organism, plants have an immune system that can fight off most pathogens. So how does that immune system work? Well, I told you about effectors. Effectors are secreted to go do good things for the pathogen, to help them infect their hosts, right? But what happens is occasionally those effectors trip the wire and activate immunity. Because plants have evolved receptors that can detect these effectors. As a metaphor, detecting effectors is a little bit like the metal detectors at the airport. If you're trying to get a gun through security, you'd be in trouble because the gun will trigger the metal detectors. That's what plants have: they have very effective immune receptors that are essentially acting as effector detectors. And, just like a gun would activate airport security, plant immune receptors detect things that look like pathogens or pathogen-related modifications.

When those immune receptors are activated, then the plant will mount an immune response. It's very effective at defending against all types of pathogens, whether they're bacteria, fungi, viruses, insects, nematodes, etc. Plant breeders have been using this for years. The products of these immune receptors are encoded by what we call *R* genes. *R* genes are basically disease resistance genes. They've been used in plant breeding for over a century.

lants have an immune system! They are resistant to most pathogens because effectors often "trip the wire" and activate immunity.

GMO blight-resistant potato expected to help over 300,000 farmers in Uganda.

Breeding *R* genes into plants takes several years, and sometimes can't even be done while retaining all the good genes that make the crop plants so productive. These days we can also use GMO transgenic systems to move *R* genes from one plant to another. This is, for example, a project from our colleagues at the Two Blades Foundation and my colleague Jonathan Jones at The Sainsbury Lab where they took three different genes that function against *Phytophthora* potato blight, and introduced them into potato. That potato is doing quite well, as you can see here, without any spraying or any chemicals to kill the pathogen. In one project, working with the International Potato Centre, known by its Spanish initials CIP, they developed the 3R potato for farmers in Uganda

where potatoes are grown not just for income but also as a subsistence crop.

ceived the image of the potato farmer from Dr. Tarlochan Thind, Punjab ricultural University. He is quoted in this story about potato late blight in India.

This problem of potato blight and other crop diseases is quite serious not just because of crop losses but also because fungicide use is something we tend to ignore a little bit, and it's very close to my heart having grown up in a developing country, Tunisia. Although crops are resistant to some pathogens, when you have a new race of pathogen that pops up and overcomes the resistance we breed, fungicides are pretty much the only solution to manage it. Fungicide use is really, really bad, not just for the environment, but also for the farmers. Farmers suffer from using fungicides, because in developing countries, they typically cannot read the labels. They often don't know what they are doing with these chemicals. They don't wear protection. You can see that in this picture from a colleague in Punjab, India. This is a potato blight field that is destroyed. It's too late to spray it. There is really no integrated pest management here. This farmer, sadly enough, lost the crop. He shouldn't be bothering spraying it, and he certainly should be wearing better protection, etc. Therefore, delivering genetically resistant plants is really important.

And it's not just in Punjab. The same is happening all over the world. Here is a potato farm worker in Venezuela. Look at that. No protection whatsoever. One comment on Twitter stated, "at least he has boots." As one Ugandan farmer who adopted the blight resistant potatoes said, "My interest in 3R Victoria is my health condition in the long term and saving money due from labor and purchase of chemicals."

**Genome editing: delivering genetically resistant plants**

Delivering genetically resistant plants is important, but breeding takes a long time and GMOs cost a lot of money—millions of dollars—to get through governmental regulation. Now what? Last year the Nobel Prize in Chemistry was awarded to two wonderful scientists, Emmanuelle

Charpentier and Jennifer Doudna, whom you can see here. They discovered CRISPR, a system that allows easy gene editing. If you think about the genome as a book, gene editing is like a word processor. It allows you to go in and change a few letters, or even remove them. You can remove sentences from your book or modify the text pretty much as you wish.

Emmanuelle Charpentier and Jennifer Doudna.

As far as biology goes, CRISPR is as much of a craze as you can get. The Charpentier and Doudna paper dates back to 2012, and very soon after that, all of us started using CRISPR. In August 2013, just a year after that Nobel Prize–winning paper came out, several of us showed that we can use CRISPR to modify plant genomes. That was one of the most exciting moments in my career. If you're a geneticist, re-writing any letter in the genome is as good as it gets.

CRISPR accelerates plant breeding cycles.

So how can we use CRISPR toward plant health? I told you about these effectors, right? Effectors have targets in the plant. If you think about the metaphor of a key and a lock, the target is like a lock and the pathogen effector is the key. If you can modify the lock or even remove the lock, the key won't work. Essentially that's the same thing. These host targets are facilitating infection, acting as what we call susceptibility factors. If you have a lock and your thief has a key, then that lock is helping the thief enter your house.

What we can do with CRISPR is modify the targets of these effectors so that the pathogen can no longer open that particular lock. This is something that we and others have done. For example, in this case, we went into tomato and removed 48 or 49 letters from the genome of this

CRISPR crops — removing genes for fungal resistance.

tomato. The text on the top is the genome of the wild type or the tomato plant we started with, and the gap there is what we removed, a very precise modification. What that modification does is make that tomato resistant to the powdery mildew fungus. This was a proof-of-concept study, but this concept of what we call susceptibility genes, a little bit the mirror image of the resistance gene, is applicable to many diseases these days. It's good stuff. That mildew resistant tomato—we called it Tomelo—is a big deal. Most growers who spray fungicides on tomatoes in the UK do so to manage powdery mildew.

Gene editing is different from transgenics—transgenics and GMOs are about adding a gene to the plant, but in this case, we're removing part of a gene. There's a lot of debate about how this could be regulated. Europe is more conservative than other places in the world. Even Japan, not exactly a cowboy nation, has recently approved a bioedited tomato for commercial production. Regulators in the US, for example, have been more open to this technology, so companies in the US are also using CRISPR. For example, my colleague Zach Lippman at Cold Spring Harbor made this tomato, which has mutations in three different genes, making it small and compact. This 'bushy tomato' is ideal for urban gardening and for other very specific niches, such as indoor cultivation. That's what's fascinating about gene editing. There's a lot of creativity going on using CRISPR to do a lot of different things, especially when the resulting plants are deregulated or treated like plants produced by classical breeding, as is more or less the case now in the US and a growing list of countries.

But guess what? Shunyuan Xiao at the University of Maryland wanted to grow this tomato and engineer it with his students, and they did that just last summer. And guess what? It just got powdery mildew! Zach's tomato actually got infected in Maryland with powdery mildew! The next iteration now is to add another mutation into this tomato to help it resist powdery mildew by inactivating a gene that makes it susceptible. And they can easily do that because the system we published for powdery mildew resistance is free, it's open science. Just like in open source

SPR bushy tomato for urban gardeners. But still susceptible to mildew.

software development, we can now share snippets of knowledge about various genes and other scientists can freely use them and combine them to breed improved crop plants.

In this example, you can see how exciting CRISPR is, because this tool allows you to design a tomato that is ideal for your needs. And if you have a problem with a particular disease, you might be able to solve it. Considering that this technology was only invented in 2012 or 2013, it's remarkable that we can already do this sort of thing and address real world problems with this CRISPR tech.

In conclusion, I'd like to remind you that although 2020 was overtaken by the COVID pandemic, we need to keep in mind the idea that, especially in developing countries, food and agriculture and plants are really important and critical to the welfare of mankind. This is nicely summarized in a quote that I borrowed from my colleague Cristobal Uauy at the John Innes Centre: "Medicine can cure you one day, but plants save our lives every day." As plant pathologists, we aim to help save plants' lives, so they can save us every day.

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The United Nations has declared 2020 the International Year of Plant Health (IYPH). In this timely talk, Prof. Sophien Kamoun introduces you to the secret life of the parasites that colonise plants. Ever since Heinrich Anton de Bary called the microbe that causes the potato blight a plant killer, we have learned much about how these microbes cause disease and fight off the plant immune system. Some of these plant pathogens even turn their plant hosts into living puppets or Zombie plants. Others are threatening our crops and driving the global food crisis. Plant pathologists like Sophien Kamoun are hard at work learning more about these parasites and applying new knowledge and technologies to build disease-resistant crops.

The speaker for this talk was Professor Sophien Kamoun, Senior Scientist at The Sainsbury Laboratory and Professor of Biology at The University of East Anglia.

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"Keeping up with the plant destroyers" is the title of this PlantVillage blog post.