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# New approaches for protecting potatoes against late blight

**Late blight continues to result in major losses and a considerable use of pesticides in potato farming. Research is under pressure to improve existing control strategies and develop new solutions. A combination of these approaches could enable potato farming in Switzerland to generate greater yields and become more ecological.**

The potato is the third most important arable crop in Switzerland after wheat and sugar beet. In 2016, it was grown on around 11,000 hectares with a yield of approximately 30 tonnes per hectare (Swisspatat, 2017). The level of self-supply for potatoes in Switzerland is more than 90 percent, higher than for any other crop plant. Yet potatoes are not only grown for human consumption, but also as animal feed and as a raw material for industrial applications.

One of the biggest challenges for potato farming is late blight, a disease caused by the *Phytophthora infestans* pathogen. Although the disease has been known for a long time and has been well researched (Box 1), it is still a cause for concern in all growing regions. If the atmospheric conditions are moist and warm (i.e. average daily temperature above 10 °C) and thus beneficial for the development of the pathogen, it can result in the loss of an entire harvest if no countermeasures are taken. Furthermore, an increase of just one degree in the average temperature, combined with high atmospheric humidity, significantly increases the pathogen's potential (Andrade et al., 1997/98), which is very alarming in the context of global warming. On average, *Phytophthora* destroys around 16 percent of global potato production every year. The losses and expenditure on measures to control the disease generate annual costs within the EU of up to 1 billion euros (Haverkort et al., 2008).

The most common sources of infection in Switzerland are infected seeds, dumps of old potatoes and secondary growth of potatoes i.e. growth of individual plants during the next season (Agroscope, 2017). As the development cycle of the pathogen is only around 3–5 days, the disease can spread rapidly throughout a region if the weather conditions are favourable. Since Agroscope started recording such data in 1990, Switzerland has suffered from late blight epidemics every year. However, the extent of the epidemic varied strongly from one year to the next; for example, there were cases of late blight in 2013 and 2015, but these did not have any major effects (information: T. Musa-Steenblock, Agroscope).

## Box 1: A plant disease that wrote history

The potato was introduced into Europe in the second half of the 16th century. The decorative flowers and (poisonous) berries were the initial source of interest. It was only gradually that the focus moved to the significance of the tuber. Potatoes were grown in Ireland from around 1700, as they thrive on barren ground. The potato subsequently became a dominant food staple on the island.

The pathogen that causes late blight, *Phytophthora* infestans, was at some point introduced into the USA through an infected plant from Mexico and then later into Europe. The disease was first recorded on the east coast of America in 1843. One year later it had reached Belgium and England, from where it spread throughout Europe. Between 1845 and 1852, the pathogen essentially destroyed the entire potato harvest in Ireland in several consecutive years and, in combination with other factors, this had devastating consequences. Between 1844 and 1851, the population on the island declined from around 8.4 million to 6.6 million. Around one million people died of hunger or, in their weakened condition, fell victim to typhoid or other diseases; a further million moved to the USA. The catastrophe was entered in the history books as the “Great Famine.”

Experts very quickly started to investigate the plant disease, and the causative pathogen was described in detail for the first time in 1845. Yet it took a further hundred years before the development cycles and mechanisms of the disease were properly understood (Schumann, 1991).

There are various strategies available for reducing losses caused by *Phytophthora* in potato farming. Suitable measures are the use of certified and non-infected seeds, the removal of potatoes from the previous season, reliable infestation monitoring, and diagnostics for early identification of epidemics. Other measures include the cultivation and growth of resistant varieties and targeted, direct control of the pathogen. There are both new crop protection products based on synthetic substances and approaches that are compatible with the requirements of organic farming. This would mean that the use of copper preparations in organic farming can be reduced.

## Breeding of resistant varieties as a basis

In potato breeding, seed potatoes fundamentally multiply through vegetative propagation (asexual). This means that seed potatoes are identical with their mother plant and the varieties remain stable. This has certain agronomic benefits, for example, all crops are ready for harvesting at the same time. However, the crops also all exhibit the same weaknesses, which plays a role in major harvest losses in the event of high susceptibility to *Phytophthora*. The breeding of new varieties can be deployed here: growing varieties with a high resistance to *Phytophthora* could save a lot of money and reduce the use of pesticides.


Trial field with *Phytophthora*-resistant and non-resistant potatoes



Symptoms on leaves, stems and tubers after *Phytophtora* infestation

Most European breeding programmes deploy classical cross-breeding, often combined with marker-assisted selection (Box 2). This entails trying to combine several resistance genes (*R* genes) from existing varieties and, at the same time, avoid the genes responsible for the susceptibility to *Phytophthora* (*S* genes, S = 'susceptibility'). It is also possible to cross additional *R* genes from wild potatoes into an existing cultivar. However, during this process many desired characteristics of the cultivar are lost and must be reintroduced via backcrossing steps. Traditional potato breeding is a lengthy process: if cultivars are crossed, it takes approximately 12–15 years to develop a marketable new variety; the process can take up to 50 years if wild potatoes are crossed.

Although there are already resistant varieties, these are not grown extensively. A significant reason for this is a lack of acceptance for the varieties in the market, as they lack some characteristics that are important for trade and for consumers. There is therefore still widespread use of crop protection products – in both traditional and bioorganic potato farming.

### Cisgenesis as a further breeding approach

Cisgenesis (Box 2) is a further possibility for introducing *R* genes into existing cultivars. Cisgenes are intrinsic genes that can be transferred within the same species or between crossable species. They therefore belong to the traditional 'breeder gene pool.' During cisgenesis, the desired *R* genes are introduced into an existing potato variety using genetic methods (Jo et al., 2014).

As the *Phytophthora infestans* pathogen continues to adapt to its environment over time, the *R* genes could lose their resistance characteristics. Since this is partly already the case and the number of *R* genes is limited, it is very important not to grow any potatoes that only contain a single *R* gene. This can prevent, or at least delay, *Phytophthora* adapting quickly and further loss of resistances. Cisgenesis makes it possible to introduce several different *R* genes into an existing variety at the same time, without this variety losing its other characteristics (Haverkort et al., 2016). During field studies, which were partially conducted on the Agroscope 'Protected Site,' the potatoes were found to be resistant to the tested forms of *Phytophthora* (Brunner et al., 2017; Haesaert et al., 2015).

Cisgenic potatoes are classified as genetically modified organisms (GMO) in Switzerland and the EU. The moratorium on genetic engineering in Switzerland bans the cultivation of GM crops until 2021. This means that cisgenic *Phytophthora*-resistant potato varieties are not permitted to be cultivated in Switzerland. In early 2017, three varieties of cisgenic potatoes were approved for cultivation in the USA into which, among other genes, an *R* gene had been introduced (FDA, 2017).

In addition to cisgenesis, other breeding techniques are used to develop potatoes that are resistant to *Phytophthora*. For example, efforts are being made to deactivate the *S* genes responsible for the susceptibility using RNAi or CRISPR/Cas9 (Sun et al., 2016). However, in contrast to cisgenesis, these approaches are still in the early stages of development (Box 2).

## Synthetic fungicides

Traditional agriculture, which accounts for the largest part of Swiss potato production, today primarily uses synthetic fungicides for controlling late blight. A combination of several preparations is mainly used to tackle the disease successfully, and these must be applied 7–8 times per year. The different fungicides are suitable for the different development stages of the potato plant and pathogen respectively. The preparations differ in terms of their mode of action, which not only increases their effectiveness, but also delays the risk of *Phytophthora* adapting to the fungicide. However, the effectiveness diminished for substances in the important class of phenylamides. There must therefore be strong restrictions in the use of these crop protection products. It is probably merely a question of time until *Phytophthora* adapts to further substance groups. There is therefore significant pressure to explore new substances and develop new preparations. However, the process of developing new fungicides takes around the same length of time as breeding new varieties. It thus takes at least 10 years to develop a new synthetic preparation.

## Box 2: Techniques for breeding potato varieties

During traditional crop breeding using conventional cross-breeding processes, the newly-generated crops are selected on the basis of their characteristics (phenotype). In contrast, **marker-assisted selection** (MAS) is based on the genetic characteristics (genotype). MAS makes it possible to select suitable parent plants before the cross-breeding process and, shortly afterwards, identify seedlings with the desired characteristics, based on their genotype. For example, it is possible to determine whether a specific *R* gene is present without having to perform a resistance test. This significantly speeds up the breeding process.

During **cisgenesis**, genes are transferred from the same or closely related variety that is compatible for cross-breeding purposes. This is the main difference from transgenesis, in which a gene foreign to the species is introduced. In terms of *Phytophthora* resistance in potatoes, this method can be used to introduce known *R* genes into an existing variety without changing their other characteristics, which is not possible with cross-breeding.

During **RNA interference (RNAi)**, specific DNA sequences are introduced into plants as transgenes to suppress production of a certain protein, which is encoded by the intrinsic plant mRNA. In potatoes this mechanism could be used, for example, to regulate proteins that are responsible for susceptibility to *Phytophthora*.

For a few years, **CRISPR/Cas9** technology and other similar methods have made targeted genome editing possible. Specific DNA sequences can be deleted, amended or added. In terms of late blight, it could be possible to suppress the *S* genes responsible for the susceptibility in the same way as with RNAi. The fact that this technology works in potatoes was shown in early 2017 when the production of starch in potatoes was changed using CRISPR/Cas9 (Andersson et al., 2017).

Synthetic fungicides must comply with regulatory conditions and are subject to strict conditions of use. Despite these stringent requirements, synthetic crop protection products can have negative effects on non-target organisms, either through direct contact or leaching into the surrounding waters. In the 'Action Plan for Risk Reduction and the Sustainable Use of Crop Protection Products' from 2017, the Swiss Federal Council outlines its intention to reduce the risks from crop protection products by 50 percent in the long term. Measures for reducing the use of fungicides in potato farming should also be examined (Swiss Federal Council, 2017).

## Non-synthetic crop protection products

In 2016, production on approximately 5 % of potato fields was in line with bioorganic standards (BioSuisse, 2017). Synthetic crop protection products are not used in organic farming, and treatments with copper preparations are currently the only effective method against late blight. However, the heavy metal accumulates in the ground and is poisonous for many organisms. In years with a high *Phytophthora* infestation rate, the maximum permissible application quantity of 4 kg of copper per hectare is quickly reached. Under such conditions, efficient control of late blight is only possible through optimised application. However, the permitted quantity of copper may only delay the spread of the disease by just a few days under such conditions. The EU is striving to ban the use of copper by 2018 (EU, 2015). The pressure to develop alternative treatment methods that meet organic farming requirements is therefore high. Such new treatment methods that could potentially be combined with synthetic crop protection products would also be of interest for conventional agriculture.

Non-synthetic substances can be found, for example, in plant extracts. Such 'botanicals' were sometimes used in agriculture 4,000 years ago. There are essentially two approaches for identifying new natural substances that could potentially be used against late blight: experimenting with available raw materials or systematically analysing collections of natural substances. An interesting source of raw materials is forest by-products (e.g. tree bark), as they are readily available and rich in substances with an antimicrobial effect. An initial product, based on a substance from larch tree bark, is already on its way to being introduced into the market. Furthermore, in field tests a suspension of bark from the black alder tree displayed a protective effect per hectare that is comparable with 3kg of copper. The second approach, namely systematically analysing entire collections of substances, has become much simpler and faster thanks to modern laboratory technologies. However, substances that delivered promising results in the laboratory or greenhouse often failed to show a sufficient impact under field conditions (Dorn et al., 2007; Krebs et al., 2013).

Interestingly, combining specific plant extracts with special fertilisers containing phosphorus and potassium could significantly enhance their effect. These special fertilisers also showed good results in field trials when used on their own. To ensure that they are effective they must be applied several times, in the same way as copper or other fungicides. However, depending on the quantity used, phosphonic acid residues are found in the harvested potatoes, which is why such potassium phosphonate preparations are not currently approved for organic potato farming. Nevertheless, new research results give cause for hope that, through the balanced application of phosphonates and potentially in combination with plant-based preparations, the use of copper can be significantly reduced (Krebs et al., 2013).

## Use of the potato microbiome as a new approach

One approach to tackling *Phytophthora*, which has received relatively little consideration to date, is the use of the potato microbiome i.e. all microorganisms that colonise the plant. As it involves the use of biological resources, this approach is also compatible with organic farming guidelines (Hunziker et al., 2015). There are essentially two mechanisms. On the one hand, other microorganisms can be supported or deployed in a targeted manner to compete with the late blight pathogen and thus inhibit its growth or reproduction. On the other hand, individual substances emitted by the microorganisms that impair the development of *Phytophthora* can be identified and used in the form of a preparation. More than 100 bacterial strains were identified on the leaves and root region of the potato, and their effect on *Phytophthora* was investigated in the laboratory. Some strains of the Pseudomonas bacteria genus are very interesting. They emit cyanide and, during tests, made it possible to significantly reduce the development of *Phytophthora*. Substances emitted by other bacteria, particularly sulphur compounds, are promising (Hunziker et al., 2015; DeVrieze et al., 2015; Guyer et al., 2015). The use of the microbiome has significant potential for efficiently tackling late blight in combination with substances that are less toxic than those previously used.

## Targeted use of crop protection products thanks to monitoring and diagnostics

The most commonly used synthetic fungicides and copper preparations have a protective effect. They are used in a preventative manner to protect crops against being infected with *Phytophthora*, because once an infestation has taken hold it is difficult to control. Infestation monitoring, refined diagnostics, and the use of forecasting models (in Switzerland: www.phytopre.ch) are therefore important to correctly assess the *Phytophthora* situation and be able to implement countermeasures in a timely manner.

It is also important to determine how long it is necessary to protect against *Phytophthora* to safeguard the harvest because if, towards the end of the growing period, the nitrogen is transported from the leaves into the tuber, the leaves will subsequently have no value for the crop. An infestation with *Phytophthora* at this time would no longer have any impact on the yield. It would therefore be possible to stop treatment and save on the use of crop protection products (Möller et al., 2006).

## *Phytophthora* management: a combination of different approaches is required

Despite major efforts being made around the world in the area of research and development, *Phytophthora* is still the biggest threat to potato farming. The various available control methods all have their limits, whether in terms of their effect, toxicity or application. Each control strategy must also be adapted to the local conditions. The best chances of success exist when various strategies, selected on the basis of the specific case, are combined. However, taking targeted and suitable countermeasures in a timely manner requires good prediction models and precise diagnostics concerning indispensable requirements.

Many experts believe that growing *Phytophthora*-resistant potato varieties is a more sustainable and successful measure than the use of crop protection products – whether synthetic or biological. New breeding techniques, such as cisgenesis or CRISPR/Cas9 technology, open up new approaches and could significantly speed up the breeding of resistant varieties. As well as the legal assessment (GMO or not), acceptance by society will also play a critical role here. Yet, when breeding new varieties, it will especially be important that new varieties are suitable for cultivation, trade and consumers.

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