

## **Final Report**

Utilizing phylloplanins for the control of fungal and oomycete pathogens in organic potato farming

## **Principal Investigator**

Amy Charkowski, Dept. Plant Pathology, Univ. Wisconsin-Madison

## **Summary**

Our long term goal is to provide organic potato farmers with either information on which potato varieties resist these common tuber diseases or to provide farmers with a way to reduce losses from these diseases. Surface defects and foliar fungal pathogens are the main cause of yield and quality loss for organic potato farmers. Farmers have relatively few options for control of these pathogens. Phylloplanins are anti-fungal proteins secreted onto leaf surfaces by trichome bearing plants such as tobacco. These proteins could be harvested from leaves to treat potatoes prior to going into storage or could be targets for breeding for disease resistance. We trialed phylloplanins produced by multiple plant species against fungal potato pathogens using a novel microfluidics device.

## **Background**

**Which potato diseases are common on organic farms?** During our conversations with the organic growers, we identified that there is a dire need for an organic product which is effective against potato pathogens. Currently, organic growers use plant resistance, which is not available for all crops/pathogen combinations, or copper sprays, which contaminate the environment with a heavy metal, to reduce losses due to fungal plant pathogens. Potato pathogens that affect tuber quality are of particular concern. In our on-farm trials, we have found that it is common for growers to lose 20% or more of their harvest to tuber surface blemish diseases and in our variety trials, we identified fungal disease, including early blight and potato early dying, as the most common causes of yield loss. Some of these diseases, such as silver scurf and dry rot, increase in severity during storage. Organic farmers have few or no methods to control these diseases.

**Are there anti-fungal compounds produced by plants that could help reduce losses from these diseases?** Leaf surfaces of about 30% of vascular plant species are covered with glandular secreting trichomes. These structures secrete proteins and secondary metabolites, many of which are antimicrobial. One of the types of proteins secreted, phylloplanins, are stable proteins that can easily be collected from leaf surfaces by gently agitating undamaged leaves in water. The tobacco version of phylloplanin is the main one that has been examined to date and its efficacy has only been tested against a limited number of plant pathogens.

The main goal of this project was to determine if this leaf-produced protein could inhibit growth of common fungal pathogens in culture and on plants. We trialed phylloplanins from tobacco, potato, and sunflower through this project. We found that only the proteins harvested from tobacco inhibited fungal growth and that only some fungal pathogens were inhibited.

The fungal pathogens included:

### **SILVER SCURF – *Helminthosporium solani***

**Symptoms:** The fungus that causes silver scurf kills just the top few layers of cells on a tuber, which causes the tuber periderm to turn grey. The symptoms are most evident on red and blue potatoes and are most evident when the potatoes are wet. Symptom severity worsens as the tubers are left in the soil; the longer potatoes are left in soil after vine kill, the worse silver scurf symptoms will become. Symptoms can also develop over time in storage, but because this is a very slow growing fungus, symptom development in storage will occur slowly. Healthy-appearing tubers are often colonized or contaminated with this fungus at harvest.

This disease is increasing in importance and incidence. There are no potato varieties that are resistant to silver scurf and silver scurf disease control methods are limited in effectiveness. This pathogen does not affect yields, but tubers may be rejected for fresh sale if severity is too high.



The discolored skin on the red potato and the greyish lesions on the russet potato were both caused by *H. solani*. This fungus produces pine tree-like spore structures on the potato surface and these

**Similar diseases:** Silver scurf symptoms resemble black dot symptoms. The margins on silver scurf lesions may be better defined than those of black dot lesions, but since these two diseases often occur together on the same tuber, the symptoms may be difficult to distinguish.

**Life cycle:** Tubers can become infected in the field and *H. solani* on both seed potatoes and soil can serve as inoculum. In fields with long rotations (greater than three years), seed tubers are likely to be the main source of inoculation. The fungus grows on the developing tubers, but the majority of symptom development occurs after vine kill. Dry conditions can cause severe symptoms even on young tubers.

Spores form on tubers in storage when the humidity is above 90% and the temperature is above 38F. The spores are spread through warehouse ventilation systems and can infect otherwise healthy tubers. Tubers can also become infected with silver scurf in storage through contact with diseased tubers. Tubers that are infected, but asymptomatic at harvest are common and these tubers can develop symptoms in storage or after washing just prior to sale.

The fungus grows on debris from many plants, including debris from crops commonly rotated with potato and it may survive in soil by colonizing rotation crop debris. This slow-growing fungus has only been reported to cause disease on potato tubers. It does not infect other root and tuber crops commonly grown in Wisconsin, such as sweet potato, carrots, parsnips, beets, or turnips. It grows well on many other substrates, including plant debris, wood, and paper. It can grow on wooden storage boxes and wood in potato warehouses. Most wild potato species are susceptible to silver scurf, but a few wild potato species have a lower level of sporulation when infected with this fungus.

### **BLACK DOT - *Colletotrichum coccodes***

**Symptoms:** The fungus that causes black dot causes symptoms similarly to *H. solani* (silver scurf) in that it kills just the top few layers of cells on a tuber, which causes the tuber periderm to turn grey. As with silver scurf, the symptoms are most evident on red and blue potatoes and are most evident when the potatoes are wet. The symptoms are often difficult to see on russet potatoes. Healthy-appearing tubers are often colonized or contaminated with this fungus at harvest.

There are no potato varieties that are resistant to black dot and control methods are limited in effectiveness. This pathogen does not affect yields, but farmers may have trouble selling tubers with severe disease symptoms.

The discolored tuber periderm and black dots on this tuber were caused by *C. coccodes*.

**Similar diseases:** Black dot symptoms on potato tubers resemble silver scurf symptoms. The margins on silver scurf lesions may be better defined than those of black dot lesions, but since these two diseases often occur together on the same tuber, the symptoms may be difficult to distinguish. *C. coccodes* also causes foliar and stem symptoms similar to early dying and it may play a minor part in the potato early dying disease complex.

**Life cycle:** Tubers can become infected in the field from spores that have formed on potato stems or from sclerotia that have survived in the soil. The small black dots that give this disease its name are fungal sclerotia and they are visible on stems, stolons, and tubers. The sclerotia can survive for several years in the soil and long crop rotations show little effect in reducing disease.

Unlike silver scurf, black dot does not spread easily in potato warehouses. Tubers that are infected, but asymptomatic at harvest are common and these tubers can develop symptoms in storage or after washing just prior to sale. Surprisingly, day length may affect black dot severity, which could explain some of the conflicting reports on the importance of this disease. Black dot is more severe under short days than long days.

## **VERTICILLIUM WILT or POTATO EARLY DYING – *Verticillium species***

**Symptoms:** This soil-borne fungus causes plants to wilt and die earlier than they otherwise would. The fungus produces a toxin that appears to play an important role in virulence and it also grows in the vascular system of the potato, which affects plant health. Resistance genes are available for this disease, but they are not commonly found in cultivated potato varieties. This fungus acts synergistically with the lesion nematode to cause potato early dying.

**Life cycle:** This fungus is common in soil and potatoes become infected as they grow in infested soil. The fungus colonizes the potato vascular system, causing the plants to wilt and die in late summer. The fungus over-wintering structure, called microsclerotia, forms in potato stems and is released into the soil as the stems decay. Removal of potato stems from fields is effective in reducing the incidence of this disease because it reduces inoculum levels.



Typical potato early dying symptoms include leaf chlorosis and wilting (Photo

### EARLY BLIGHT - *Alternaria species*

**Symptoms:** This fungus causes necrotic lesions on potato leaves, stems, and tubers. As the lesions become larger, they form a characteristic bullseye pattern and the lesion may be surrounded by a chlorotic halo. The disease can be severe on organic farms and often causes significant yield losses.

**Life cycle:** The fungus overwinters on crop debris in soil or on alternate hosts or volunteer plants. Disease inoculum comes from plant debris or spores spread by wind or rain splash. This disease is more severe when humidity is high, so it often becomes more significant as the growing season progresses and the potato canopy closes.



The necrotic lesion on this potato leaf was caused by *Alternaria*. (Photo from Amanda Gevens)

### DRY ROT - *Fusarium sambucinum*

**Symptoms:** This fungus causes dark lesions in tubers that are filled with a dense mass of fungal hyphae. The tuber skin may stay almost entirely intact, but will appear shriveled or dented above the dry rot lesions. The lesions can become large, killing the entire tuber.

**Life cycle:** The fungus can survive in soil for years. Since it requires a wound to infect a tuber, it usually only rots cut seed pieces at planting or infects tubers as they are harvested. The disease will progress in storage and

secondary invaders, such as soft rot bacteria, can exacerbate problems caused by dry rot.

**Objectives:** Our goal with this project is to determine if harvested defensive leaf surface proteins from trichome bearing plants such as tobacco, cultivated and wild potato, tomato and sunflower can effectively inhibit pathogens of potato that cause significant losses to organic potato growers. We conducted *in vitro* spore germination assays using phylloplanins which were collected by gently agitating undamaged leaves in water, lyophilizing the resulting solution and re-suspending the lyophilized product in sterile water. We also developed a microfluidics assay to simplify trialing natural products for inhibition of plant pathogens.

**Results:** The inhibition of spore germination by various phylloplanins was tested against four potato pathogens: *Alternaria alternata* (early blight), *Verticillium albo-atrum* (potato early dying), *Fusarium sambucinum* (dry rot) and *Colletotrichum coccodes* (black dot). We also conducted assays with *Helminthosporium solani* (silver scurf) but results were inconsistent and difficult to interpret. Phylloplanins collected from potato, tomato, and sunflower did not inhibit spore germination of any of the pathogens tested. But, tobacco phylloplanins strongly inhibited the spore germination of *C. coccodes* and transiently inhibited *F. sambucinum*. The tobacco phylloplanins had no detectable activity against *A. alternata* or *V. albo-atrum*.

*C. coccodes* spores suspended in solutions containing tobacco phylloplanins did not germinate at 48 hours post treatment. After washing the spores, they still did not germinate, therefore the inhibition appears to be irreversible. *F. sambucinum* spores germinated in negative controls at 12 hours post inoculation (hpi), but not in the presence of tobacco phylloplanins. However, at 24 hpi, this inhibitory effect was no longer evident.

From our data, we concluded that tobacco phylloplanins have a spectrum of antimicrobial activity and that host-pathogen co-evolution may select for resistance to phylloplanins in some fungi. Of the fungi we assayed, they are likely to only be useful to control *C. coccodes*. Further studies on the ease of acquisition of resistance to phylloplanins would aid in decisions on whether to develop these antimicrobial proteins as a natural product for use in plant protection.

We are preparing a publication that describes these results and the microfluidics assay.