Potato Progress

Research & Extension for the Potato Industry of Idaho, Oregon, & Washington

Andrew Jensen, Editor. ajensen@potatoes.com; 509-760-4859

www.nwpotatoresearch.com

Volume XVII, Number 3 | *13 March 2017*

New Approaches to Root-Knot Nematode Resistance

Cynthia Gleason, Department of Plant Pathology, Washington State University

Columbia Root-Knot Nematode (CRKN, *Meloidogyne chitwoodi*) is a serious pathogen of potato, infecting both roots and tubers, in the latter causing pimple-like blemishes and brown spots in the flesh. These quality defects make the infected tubers unacceptable for fresh markets and processing. CRKNs are well-adapted to temperate climates, like those found in the Pacific Northwest, and they become active at relatively low temperatures. This means that the population will grow rapidly during long, warm growing seasons (Pinkerton et al., 1991). Unless CRKNs are controlled, the presence of just one juvenile per 250 g of soil at the beginning of the growing season could lead to large nematode populations (several thousand/ 250 g soil) by harvest, which undoubtedly will lead to significant tuber damage and yield loses (Ingham et al., 2000).

There is an urgent need to reduce reliance on fumigants and other pesticides for nematode management. With this in mind, I joined the Plant Pathology Department at Washington State University in March 2016 and started a research program in plant parasitic nematodes with a specific focus on CRKN. I have worked on root-knot nematode for several years. I received a Ph.D. from U.C. Davis, where I studied root-knot nematode genes, and just prior to moving to Pullman, Washington, I was a Junior Professor in Germany, where my group worked on root-knot nematodes and the molecular plant responses to infection (Gleason et al., 2016; Gleason et al., 2017). CRKNs are a quarantine pathogen in Europe, so work on this particular root-knot nematode was not possible in Germany. I am quite excited to be at WSU and extend my research to a species of root-knot nematodes that is very important to potato growers in the region.

Natural resistance provides growers with an environmentally friendly (and potentially less costly) means of nematode control. In fact, a single dominant resistance (R) gene called $R_{Mc1(blb)}$ was introgressed from a wild species of Mexican potato (*Solanum bulbocastanum)* into an elite potato cultivar; the subsequent backcrossing into the cultivated potato gene pool has resulted in a clone called PA99N82-4 (Brown et al., 1995; Mojtahedi et al., 1995). However, one major shortcoming is that nematode populations have a habit of changing so that they overcome the plant resistance. Already a $R_{Mc1(blb)}$ resistance-breaking nematode pathotype of *M. chitwoodi* (called WAMCRoza) has been isolated from a field containing resistant breeding lines (Brown et al., 2009). As a result, R-gene mediated resistance alone may not be a durable, long-term solution to nematode management. With this in mind, my lab studies what happens in the plant and nematode when the nematode is successful in establishing itself in the plant; this is called the susceptible response. Although diseased plants are obviously not the goal of the grower, in my lab, we are using the CRKN-infected plants to ask a question – what does the nematode need from the potato plant in the susceptible response?

One of the most critical things that the nematode needs from the plant is food. Upon entering the plant, the nematode migrates to the root vasculature and establishes its feeding sites. The nematode converts up to 10 plant cells into feeding structures called "giant cells." The nematode cannot survive without these giant cells. Therefore, we are interested in identifying plant genes that encode proteins necessary for the nematode's success in the plant and that are involved in giant cell formation or maintenance. A loss-of-function or mutation in these critical "susceptibility genes" can lead to a resistant plant (van Schie and Takken, 2014).

There are over thirty thousand genes in potatoes, so figuring out which genes are needed for the susceptible response and that do not alter normal plant development is a daunting task. To approach this task, my research team uses the nematode as our guide. During infection, the nematodes produce secretions that are injected into the plant. These secretions are sometimes referred to as the nematode "spit." Scientists will refer to the spit proteins as "effectors" because they have an effect on the plant – either 1) a suppression of plant defense responses and/or 2) an assistance in establishing the feeding sites. They are very "effective" in helping the nematode infect the plant. We plan to identify and study CRKN nematode effectors and identify the susceptibility gene products in the plant that the effectors are targeting (Figure 1).

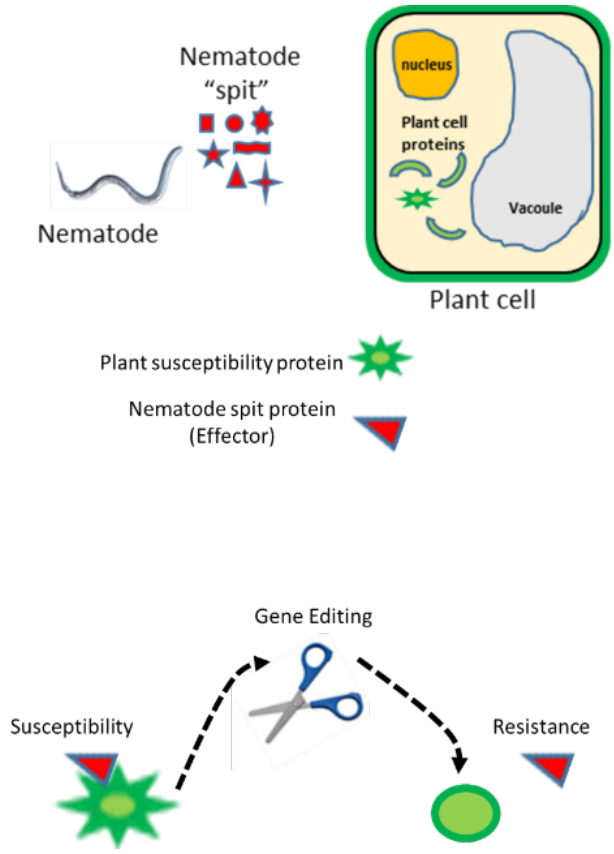


Figure 1. A simplified representation of nematode and plant interaction. The nematode secretes several proteins into the plant during nematode infection. These proteins are referred to as "effectors" (represented by red symbols). My research will investigate the nematode effectors. These effectors may interact with plant susceptibility proteins (represented by green star), leading to the susceptible interaction. Gene editing can be performed to modify the plant. Consequently, the susceptibility plant protein is different and the nematode effector can no longer interact with it. As a result, the plant will have nematode resistant characteristics.

For the effector-led approach to susceptibility gene identification, we established cultures of CRKN Race 1 (WAMC1), Race 2 (WAMC2), and WAMCRoza, and developed an efficient potato infection system in sand. WAMC1 and WAMC2 differ in their ability to reproduce on alfalfa and carrot (Mojtahedi et al.,

1988). WAMC1 and WAMCRoza are two pathotypes that differ in their virulence on potato roots that carry the $R_{Mc1(blb)}$ resistance gene. We are currently collecting infected potato tissue in order to capture nematode gene expression during the initial stages of infection and feeding. My team has confirmed that PA99N82-4 is resistant to WAMC1, but is susceptible to WAMCRoza (Table 1). Early after inoculation of PA99N82-4 with the avirulent (WAMC1) and virulent (WAMCRoza) nematodes, we observed root swellings. By staining the roots with acid fuschin, we determined that the swellings coincided with root areas that contain juvenile nematodes. In susceptible plants, these swellings become small "galls," which surround the adult nematodes. However, in the resistant response, the root swellings do not become visible galls because the nematodes do not survive in the resistant roots. The observation that the roots slightly swell during early infection, regardless if it is the resistant or susceptible interaction, has allowed us to collect nematode-enriched root tissue in both the resistant and susceptible plants for subsequent transcriptome analysis. We obtained nematode-enriched galls and we will study the nematode genes that are expressed in these galls. From these samples, we will obtain data about the nature of the effector complement during the potato-nematode interaction. These data will provide us with effector candidates to use in our plant susceptibility gene search. This work will also give us information about the genes in the different CRKN races. Using this information, we want to establish molecular markers so that we can distinguish CRKN WAMC1, WAMC2, and WAMCRoza using easy, fast tests in the lab, such as PCR (Polymerase Chain Reaction).

My lab's long term goal is to develop resistant potatoes that will reduce reliance on soil fumigants and nematicides. To achieve this goal, we want to exploit plant susceptibility genes that encode plant proteins. We want to target genes for modification so that genes that critical to the success of the pathogen are now absent. To overcome this, the nematode would need to gain new gene activity. This unlikely event means that modified disease susceptibility genes are more likely to provide durable, broad-spectrum resistance.

We can modify the potato genome in several ways. For example, we can try to identify traits in wild species of potato and introduce those traits into the cultivated potatoes. However, this process can take several years, and as we have seen with $R_{Mc1(blb)}$, the durability of such genes can be questionable. Another approach is to make small changes in the genome in a process called genome editing (also referred to as CRISPR-Cas9). It allows us to make cuts in DNA that lead to mutations in specific genes. These mutations could occur in nature if we waited around long enough in the evolutionary time scale, but we now have the tools to speed up this process. Small changes to the potato genome would allow us to selectively "turn off" the genes required by the nematode when they try to establish a feeding site. This technology will revolutionize plant biotechnology because it has the potential to be used to enhance plant resistance to parasitic nematodes and many other plant pests in a wide variety of crops.

Table 1. Interaction between potatoes and CRKN nematodes. Depending on the potato and the race of nematode, the response in the plant can be a susceptible (disease present) or resistant (no disease).

	Nematode		
Potato	WAMC1	WAMC2	WAMC Roza
Russet Burbank	Susceptible	Susceptible	Susceptible
PA99N82-4 potato with *RMc1(blb)*	Resistant	Resistant	Susceptible

Brown, C.R., Mojtahedi, H., and Santo, G.S. (1995). Introgression of resistance to Columbia and Northern root-knot nematodes from *Solanum bulbocastanum* into cultivated potato. . *Euphytica* 83**,** 71-78.

Brown, C.R., Mojtahedi, H., Zhang, L.H., and Riga, E. (2009). Independent resistant reactions expressed in root and tuber of potato breeding lines with introgressed resistance to Meloidogyne chitwoodi. *Phytopathology* 99(9)**,** 1085-1089. doi: 10.1094/PHYTO-99-9-1085.

Gleason, C., Leelarasamee, N., Meldau, D., and Feussner, I. (2016). OPDA Has Key Role in Regulating Plant Susceptibility to the Root-Knot Nematode Meloidogyne hapla in Arabidopsis. *Front Plant Sci* 7**,** 1565. doi: 10.3389/fpls.2016.01565.

Gleason, C., Polzin, F., Habash, S.S., Zhang, L., Utermark, J., FMW, G., et al. (2017). Identification of two *Meloidogyne hapla* genes and an investigation of their roles in the plant-nematode interaction *Mol Plant Microbe Interact.*

Ingham, R.E., Hamm, P.B., Williams, R.E., and Swanson, W.H. (2000). Control of *Meloidogyne chitwoodi* in potato with fumigant and nonfumigant nematicides. *J Nematol* 32(4S)**,** 556-565.

Mojtahedi, H., Brown, C.R., and Santo, G.S. (1995). Characterization of Resistance in a Somatic Hybrid of *Solanum bulbocastanum* and *S. tuberosum*, to *Meloidogyne chitwoodi*. *J Nematol* 27(1)**,** 86-93.

Mojtahedi, H., Santo, G.S., and J.H., W. (1988). Host tests to differentiate *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla* 1. *J Nematol* 20(3)**,** 468-473.

Pinkerton, J.N., Santo, G.S., and Mojtahedi, H. (1991). Population dynamics of *Meloidogyne chitwoodi* on Russet Burbank potatoes in relation to degree-day accumulation. *J Nematol* 23**,** 283-290.

van Schie, C.C., and Takken, F.L. (2014). Susceptibility genes 101: how to be a good host. *Annu Rev Phytopathol* 52**,** 551-581. doi: 10.1146/annurev-phyto-102313-045854.

How to Use the Research Library at http://www.nwpotatoresearch.com/

Andy Jensen, Manager
Northwest Potato Research Consortium

Below is an excerpt from the first page, for the remainder of the help guide, login to the Research Library and click on one of the Help Guides buttons download the full PDF.

During 2016 the Northwest Potato Research Consortium unveiled a new website, including a completely re-programmed "Research Library." This database of articles and newsletters includes more than 1,400 PDFs, almost all of which are full-text searchable. Material in this database goes back to the early 1960s, and it is added to regularly. Using the library is simple, but a few instructions and illustrations may be helpful. With this instructions document I hope to give you enough information to put you in the right direction toward fully utilizing our database. Please note that our policy is to grant login access to members of the U.S. potato industry and the U.S. research community. The screen shots below are from a desktop PC computer. The website is fully mobile-device compatible, but the exact appearance of each screen will depend on size and type of device you use.

Go to http://www.nwpotatoresearch.com/ for the rest of this help guide.

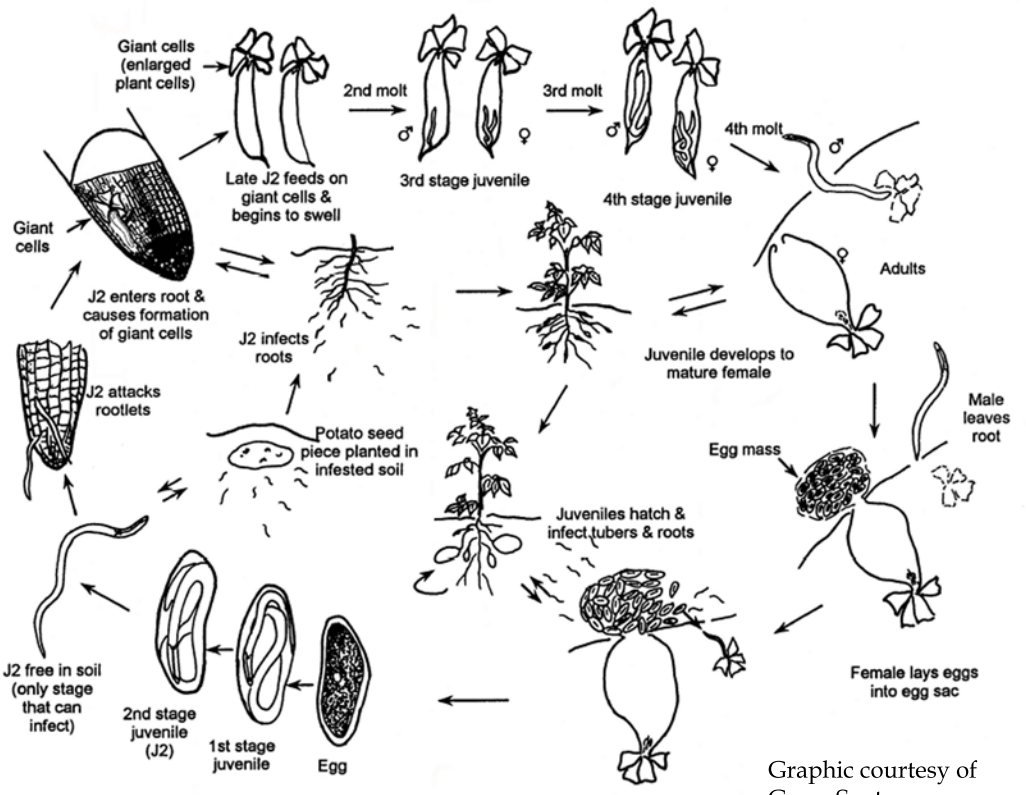
Root-Knot Nematodes

See also: http://www.nwpotatoresearch.com

(208) 334-2350

(509) 765-8845

Root-knot nematodes have a complex life cycle.



Graphic courtesy of Gerry Santo.

General Information

Nematode species: *Meloidogyne chitwoodi* (Columbia root-knot nematode), *Meloidogyne hapla* (northern root-knot nematode)

Biology: These plant-parasitic nematodes have complex life cycles (see above) involving a mobile stage that invades plant roots and tubers, and sedentary stages embedded in plant tissue. Root-knot nematodes overwinter easily throughout the Northwest. Most live in the top two feet of soil, but sometimes they are found up to 6 feet deep.

Distribution: Both northern- and Columbia root-knot nematode are widely distributed across the western states of the U.S. In the Northwest, Columbia root-knot nematode is most prevalent and damaging.

(503) 239-4763

2017 WA Commercial Potato Seed Lot Pick up &Trial Information

Info also available each year at: **www.potatoes.wsu**

Commercial potato seed samples are requested from WA Growers for the 2017 Washington Seed Lot Trial. **Two hundred whole (single drop) seed is an acceptable sample size, or 50 lbs of 4 oz single drop seed.**

Requested: 50 lbs of 2-4 oz whole seed, no seed treatments
We want a representative sample - if applicable, include a representative amount of rotten tubers!

(Seed over 6 oz is not acceptable)

A representative sample is needed. Sampling the first (or last) 300 seed from the truck is not likely to provide a representative sample of the lot. Sample tags may be obtained by calling the Potato Commission at 509-765-8845.

Your assistance with collection and drop off of seed samples is needed. Seed samples may be taken to the WSU Othello Research Unit (509-488-3191); located on Booker Road ¼ mile south from State Highway 26 and about five miles east of Othello. For sample pick up and any questions regarding the seed lot trials please call:

South Basin: Tim Waters (509-545-3511), Mark Pavek (509-335-6861), or Zach Holden (509-335-3452).

North Basin: Carrie Huffman Wohleb (509-754-2011), Mark Pavek (509-335-6861), or Zach Holden (509-335-3452).

Westside: Don McMoran (360-428-4270), Mark Pavek (509-335-6861), or Zach Holden (509-335-3452).

In the North Basin, one seed "drop-off" has been established. It is located at Qualls Ag Labs (Mick Qualls, 509-787-4210 ext 16) on the corner of Dodson Road and Road 4; come to front office between 8 am and 5 pm. Please call the numbers below to arrange additional pick up sites. Samples will be picked up at 2:00 pm the day before each planting date (below) to be included. Growers planting in early March should drop their samples off at the Othello Research Center or store the samples and call the numbers below for pick up. For all alternative pick up locations or questions please call Mark Pavek at 509-335-6861 or Zach Holden at 509-335-3452.

PICK UP DATES ARE ONE DAY PRIOR TO THE PLANTING DATES BELOW

The seed lot planting dates for 2017 are:

1st (Early)	March 28
2nd	April 10
3rd	April 25
4th (Late)	May 3 (accepting seed lots until May 8)

2017 Potato Field Day - Thursday June 22

This year's virus reading of the seed lots will take place on June 6 and 20
(If plants are too small, the first reading will be moved to June 13)