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Stubby root nematodes and associated corky ringspot disease

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Abbreviations used throughout this article:

SRN – Stubby Root Nematode
TRV – Tobacco Rattle Virus

Cover photo: corky ringspot symptom on potato tubers

Stubby root nematodes (SRNs) damage plant roots, and more devastatingly, vector tobacco rattle virus (TRV), the causal agent of corky ringspot disease (cover photo) of potato. Corky ringspot is a major factor in the devaluation and culling of potato tubers in the Northwest USA. A recent field survey showed that SRNs were third in prevalence of plant-parasitic nematodes following root lesion and root-knot nematodes (Zasada et al. in review). There is little current data on the prevalence of TRV, as the last field survey was conducted over two decades ago (Mojtahedi et al. 2000). Past research has shed light on the distribution and ecology of the nematode, plant-nematode interactions (Mojtahedi and Santo 1999;

Mojtahedi et al. 2001; Mojtahedi et al. 2003), and chemical and cultural control methods of corky ringspot (Brown et al. 2000; Ingham et al. 2000; Ingham et al. 2007; Charlton et al. 2010). However, more research is warranted to obtain an updated status of TRV in the Northwest and to determine whether populations of the virus and nematode are genetically different throughout the Northwest potato growing region. Here we briefly overview the basic biology of the nematode, associated TRV, and primary management strategies of corky ringspot in the Northwest.

Fig. 1. Stubby root nematode *Paratrichodorus* sp.

The nematode

SRN is a medium sized nematode (adult is approximately 700 μm long, Decraemer 1995), though it is difficult to see without the aid of a microscope. It is transparent and colorless when observed under a microscope (Fig. 1). Stubby root nematode is the common name of plant-parasitic nematodes in the family Trichodoridae. This family contains five genera, among which *Trichodorus* and *Paratrichodorus* have worldwide distribution and the greatest number of species (Decraemer and Robbins 2007). Stubby root nematode is an ectoparasite, meaning its body remains outside of the root while feeding. The feeding often causes a stunted appearance to the root systems of plants (Fig. 2), hence the name stubby root nematode.

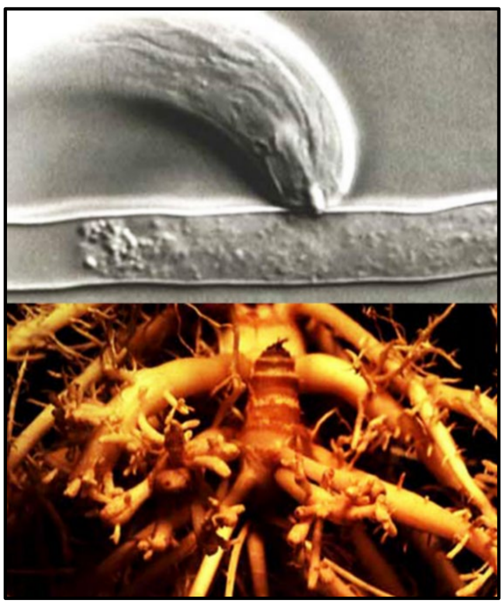

Fig. 2 Top: SRN feeding on a root hair. Bottom: Corn roots with stunted symptoms as a result of nematode feeding.

SRNs are drastically different from the other plant-parasitic nematodes that are abundant in the Northwest, namely the Columbia root-knot nematodes and root lesion nematodes. Root-knot nematodes become sedentary after they select a feeding site. Conversely, SRNs are mobile and do not feed on the root tissue at a single location, thus they are a "migratory ectoparasite" based on their feeding ecology. From an evolutionary perspective, SRNs diverged from root-knot nematodes 550 million years ago (Lambert and Bekal 2002). All plant-parasitic nematodes have a stylet, a needle like spear used by nematodes to puncture through plant cells to withdraw nutrients and secrete molecules into the plants. However, SRN has an onchiostylet (Fig. 3), setting it apart from many other plant-parasitic nematodes. The onchiostylet is curved and solid, whereas many other plant-parasitic nematodes have a straight and hollow stylet, called a stomatostylet (Fig. 3). The stylet is an important identification trait, differentiating SRN from other plant-parasitic nematodes present in the Northwest.

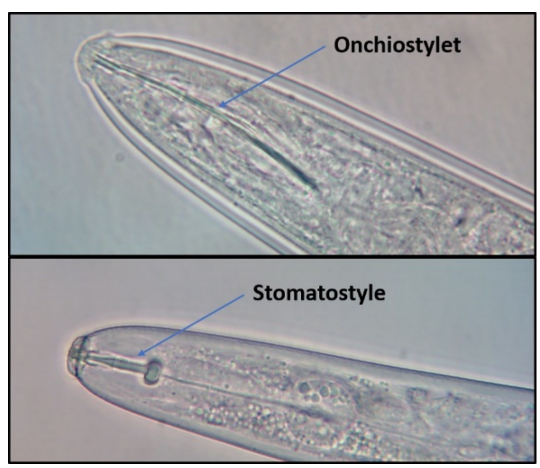

Fig. 3. Comparison of mouth parts of SRN (upper) and root lesion nematode (lower), which bear onchiostylet and stomatostylet, respectively.

The life of the SRNs starts when eggs are laid by females in the soil. They then undergo four juvenile stages, delimited by molts, before reaching adulthood (Fig. 4). SRNs are amphimictic, meaning both females and males are required to mate and produce offspring, unlike parthenogenesis, a form of asexual reproduction that is often found in root-knot nematodes. Thus, it is common to observe relatively equal numbers of female and male SRNs in soil samples. After female SRNs lay eggs, the eggs remain in the soil until they emerge as second-stage juveniles (J2) (the 1st molt occurs within the egg). Juvenile nematodes resemble adults, but are smaller in size. SRNs are obligate plant parasites, and they must utilize plant root tissue as a food source to complete their life cycles.

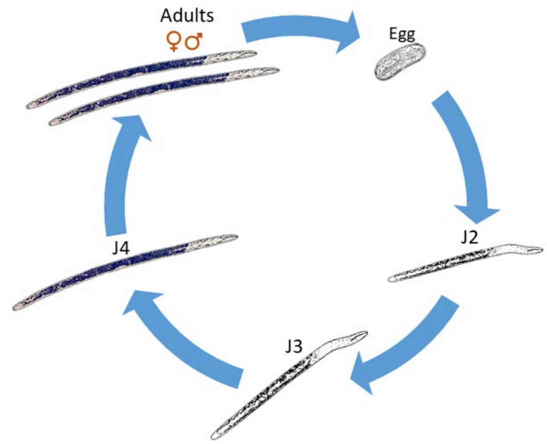

Fig. 4. Life cycle of SRN (J = juvenile).

There are currently three species of SRN reported in the Northwest: *Paratrichodorus allius* is found in 30% of soil samples (Mojtahedi et al. 2000), *Paratrichodorus teres* has occasionally been reported (Riga et al. 2007), and *Paratrichodorus minor* was recently discovered (unpublished data). Identifying plant-parasitic nematodes to species based on morphological traits has proven to be challenging. Additionally, the taxonomic skill required to identify species, e.g. the ability to tell apart *P. teres* and *P. allius,* is diminishing (Riga et al. 2007). *Paratrichodorus teres* and *P. allius* are morphologically similar, and the primary taxonomic traits for differentiating the two are only present in adult nematodes, for instance genital and vulva position (Decraemer and Baujard 1998). Often, the populations recovered from soil are comprised of morphologically indistinct juveniles, rendering identification even more challenging. A molecular-based technique is now available to identify *P. allius* and *P. teres* (Fig. 5). This technique uses PCR to amplify two sections of the 18S ribosomal RNA gene, producing distinct bands (672 bp and 432 bp) for *P. allius* and *P. teres*, respectively (Riga et al. 2007).

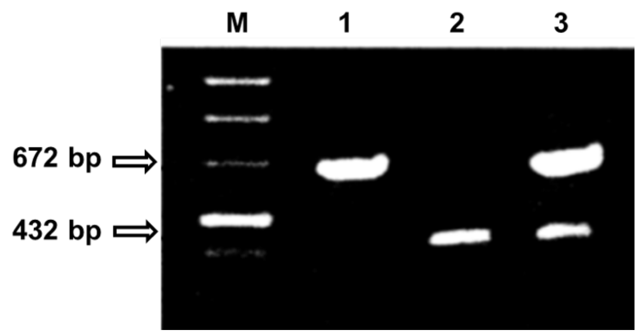

Fig. 5. PCR based technique to identify *P. allius* and *P. teres*. M: maker; Lane 1: *P. teres*; 2: *P. allius*; 3: both species (modified from Riga et al. 2007).

SRNs can undergo multiple generations in a year, and populations can build up rapidly if a suitable plant host is present. Conversely, the populations can decline quickly if the plant host is poor in nutrition, or the suitable host is removed. Therefore, sampling when host plants are still present, and populations are still high is critical to get an accurate estimate of the nematode density (Hafez and Sundararaj 2009). Data from the Pacific Northwest diagnostic laboratories from 2012 to 2016 showed that SRN can be found at very high densities in soil, 1,205 SRN in 250 cc of soil (roughly the volume of an 8-ounce cup). On average, 29 SRN were present in 250 cc of soil and 22% of the 38,022 screened soil samples contained SRN (Zasada et al. in press).

The vector and the virus

All three SRN species present in the Northwest are capable of transmitting TRV (Decraemer and Robbins 2007), a serious viral pathogen of potatoes. TRV is made of two single-stranded RNA molecules, which are wrapped within a protein shell into a rod-shaped, helical structure, called a capsid.

TRV particles vary in length; short forms range from 55 to 114 nanometers, and long forms range from 180 to 197 nanometers (Otulak et al. 2012; Fig. 6, left). Potato is considered a poor host for the nematode vector. However, potato is a great host for TRV (Mojtahedi and Santo 1999). The typical symptoms of corky ringspot range from internal flecks to large ring-shaped “corky” browning of the flesh tissue, thus the name “corky ringspot disease” (Fig. 6, right). It is also known as spraing or sprain (Hafez and Sundararaj 2009). However, TRV-caused symptoms show great variation depending on potato varieties, virus strains, and environmental conditions, thus typical corky ring symptoms might not always present. Additionally, several other viruses cause similar symptoms as TRV (Crosslin and Hamm 2008), necessitating further testing of the virus using a molecular technique (see below). Potatoes that are infected with TRV can be devalued and even rejected if over 6% of the tubers are graded as culls, causing economic loss to potato growers in the Northwest (Ingham et al. 2000). Identification of corky ringspot can be challenging given a few factors: 1) foliar symptoms are usually negligible, making it difficult to diagnose the presence of the virus based on the above-ground symptoms; 2) low population densities of SRN (0–3 *P. allius*/250 cc soil) can cause severe damage to the potato tubers if left untreated (Mojtahedi et al. 2001), and researchers have even failed to recover SRN from soil samples from potato fields where corky ringspot symptoms were observed; and, 3) the symptoms can sometimes be mistaken for internal brown spot, a physiological stress disorder.

Fig. 6. Left: TRV, the causal pathogen of corky ringspot, under electron microscope; middle: corky ringspot symptom can be negligible on potato tubers externally; right: severe corky ringspot damage of potato tuber (internal).

As SRNs migrate through the root system, the chance of transmitting the virus through the stylet to the root tissue is extremely high. Though SRN can retain the virus for a long period, it seems the virus can be lost during nematode molting, as juveniles lose virulence to infect host plants after 1-2 molts (Ayala and Allen 1968). This means that in order to pick up the virus, SRN will need to feed on infected plant tissue. Without the vector, TRV can be present in potato tubers for up to three generations of vegetative propagation (Xenophontos et al. 1998). Field-collected SRN are often comprised of a mixture of infected and non-infected nematodes. A molecular diagnostic tool can be used to detect the virus from a mixture of five SRN containing at least one viruliferous (virus-containing) individual (Fig. 7). This rapid method for virus detection circumvents the traditional technique which requires the time-consuming inoculation of an indicator plant, such as tobacco, with nematodes, followed by virus testing (Riga et al. 2009).

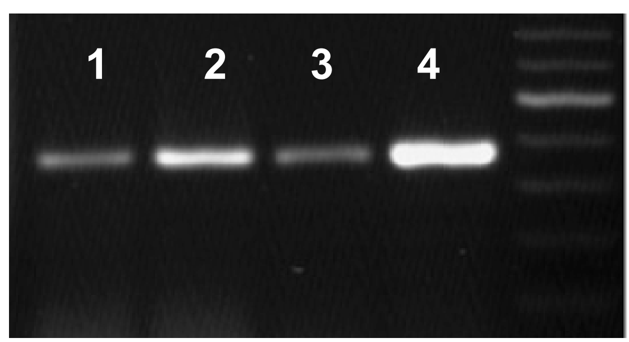

Fig. 7 A molecular diagnostic tool was developed to determine if SRN carry TRV; Lanes 1-3: DNA extracted from the equivalent of 1/4 viruliferous SRN; 4: virus DNA as a positive control (modified from Riga et al. 2009).

TRV infection largely depends on the time of infection, potato cultivars, and virus strains. There is a wide range of variation in pathogenicity among the virus strains. For instance, in a study in which three TRV strains were compared on two potato cultivars, the most severe tuber symptoms were caused by a virus strain isolated from Pasco, WA (Mojtahedi et al. 2001). Studies on timing of nematicide application suggest that newly formed potato tubers are more susceptible to the virus infection, possibly because the skin periderm is tender and could be readily penetrated by the stylet of the nematode. When the tuber periderm thickens, it might become harder for nematodes to feed and likely leads to reduced virus transmission (Weingartner et al. 1975; Charlton et al. 2010). Additional research is warranted to test these hypotheses. Finally, potato cultivars vary in their response to TRV, which forms the foundation of breeding potato cultivars that are resistant to TRV (see below "cultural management").

Chemical management

Suppressing SRN is an effective way to manage corky ringspot on potatoes. Similar to management strategies that target other plant-parasitic nematodes, nematicides have proven to be effective, including the fumigant 1,3-dichloropropene (1,3-D, the active ingredient of Telone II) and metam sodium applied prior to planting. As an effective nematicide, Temik is no longer available. Metam sodium has been reported to be more effective when shank injected (Fig. 8) rather than when delivered through irrigation water (Ingham et al. 2007). Some degree of failure in controlling SRN using 1,3-D has been documented in Klamath Basin in southern Oregon (Rykbost et al. 1995). Though the reason remains unclear, it was speculated that SRN migrate vertically in the soil, thus it is likely that nematodes moved below the fumigation zone. The non-fumigant nematicide oxamyl (Vydate) can be applied at planting or post-planting. While oxamyl may not suppress the SRN population, it significantly reduced symptoms of corky ringspot on Yukon Gold potato if applied early (earlier than 55 days after planting; Charlton et al. 2010).

Fig. 8. Tractor pulling shanks injecting fumigant (top), followed by a disk (bottom right) to close the furrows, and then followed by a packer (bottom left) to compress and seal the soil.

Cultural management

TRV-resistant potato cultivars are appealing to growers. As mentioned earlier, potatoes displayed a range of symptoms when grown in a TRV-infected field, from symptomless to severely necrotic (Fig. 9). Identifying the source of this resistance has led to the successful breeding of corky ringspot resistant varieties. Recent Tri-state releases Castle Russet, Echo Russet, and Pomerelle Russet are resistant/tolerant to TRV with good agronomic traits. These varieties show no to minimum symptoms when grown in heavily infested TRV soils while the susceptible cultivars have severely necrotic tubers. The latest release Castle Russet did not show any internal tuber symptoms and tested negative for TRV. To assure consistency when testing potato cultivars for corky ringspot resistance, the potato breeding program at USDA-ARS Prosser established two research plots, each field 0.5 ha in size, and was infested with viruliferous nematodes (Mojtahedi et al. 2007).

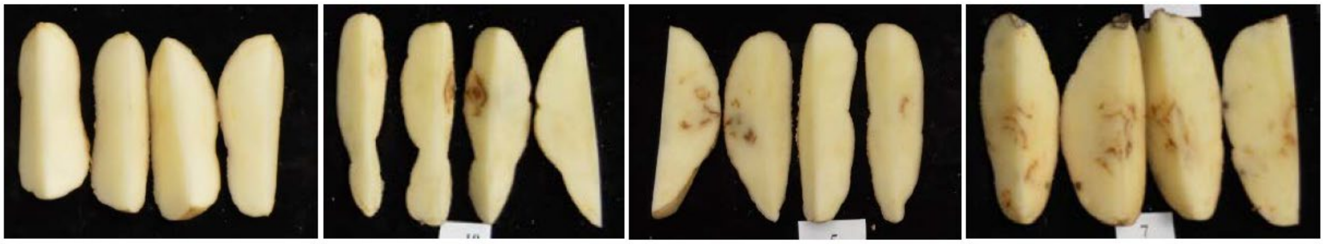

Fig. 9. From left to right: asymptomatic, mild, moderate, and severe symptoms in tubers harvested from a TRV-infested field.

Crop rotation has been an effective management strategy for pathogens with a narrow host range. Stubby root nematode can utilize a wide range of hosts, including multiple weed species and many commonly planted crops in the Northwest, such as onion, spearmint, corn, and sugar beet. Alfalfa could be a safe option for crop rotation as SRN does not feed on alfalfa (Hafez and Sundararaj 2009). Careful weed management is important when incorporating alfalfa as a rotation crop, as over half of the commonly found weed species in the Northwest are suitable hosts for SRN (Mojtahedi et al. 2003).

Future directions

A recent compilation of the Northwest diagnostic laboratory reports showed that on average 22% of the screened soil samples from potato fields in the Northwest contained SRN. The number of potato fields infected with TRV is unknown, as the last survey on TRV occurrence was conducted two decades ago. We suggest future studies could address the following research areas:

- A comprehensive survey is needed to gain a better understanding of TRV occurrence, and to examine if genetically diverse TRV strains are present in the Northwest. Dr. Hanu Pappu at WSU is leading an ongoing project which uses whole genome sequencing to scrutinize the diversity of TRV nationwide.
- As virus strains display variation in pathogenicity on potato plants, it is important to estimate the genetic diversity and pathogenicity of the virus.
- Appropriate crop rotation regimes could be effective for disease management. It is essential to know whether a field contains the virus and the nematode and the host-pathogen interactions, for instance, onion and spearmint are good hosts for SRN, yet are poor hosts for TRV.
- Finally, it is worth exploring other management strategies besides chemical nematicides, such as reducing virus transmission by disrupting the probing and feeding behavior of the nematode.

In summation, an integrated approach that incorporates various strategies should be considered to tackle this nematode-virus problem in the Northwest.

Useful resources:

Introduction to Plant-Parasitic Nematodes - https://www.apsnet.org/edcenter/intropp/pathogengroups/pages/intronematodes.aspx

Stubby root nematode - http://entnemdept.ufl.edu/creatures/nematode/stubbyroot/trichodorus_obtusus.htm

Fumigating Soils for Nematode Control - https://pnwhandbooks.org/plantdisease/pesticide-articles/fumigation/fumigating-soils-nematode-control

AGNEMA, The Phytopathology Lab - www.agnema.com

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Cover page, corky ringspot virus symptom on potato: AGNEMA

Fig. 1. AGNEMA

Fig. 2. top: Photograph by Urs Wyss, Institute of Phytopathology, Germany.
bottom: Photograph by Society of Nematologists.

Fig. 3. AGNEMA

Fig. 4. Illustration by AGNEMA

Fig. 5. Modified from Riga et al. 2007

Fig. 6. left, https://en.wikipedia.org/wiki/Tobacco_rattle_virus
middle: AGNEMA
right: Zhen (Daisy) Fu

Fig. 7. Modified from Riga et al. 2009

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https://pnwhandbooks.org/plantdisease/pesticide-articles/fumigation/fumigating-soils-nematode-control

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