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TOBACCO RATTLE VIRUS IN PEONIES: A REFERENCE GUIDE FOR CUT FLOWER AND ROOTSTOCK PRODUCERS

By
Andrea Garfinkel, Puyallup Research and Extension Center, Washington State University. **Todd Steinlage**, Alaska Department of Natural Resources, Division of Agriculture, Palmer, AK. **Janice Chumley**, Cooperative Extension Service, University of Alaska Fairbanks. **Gary Chastagner**, Puyallup Research and Extension Center, Washington State University

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What is *Tobacco rattle virus*?

Tobacco rattle virus (TRV), previously referred to as *Peony ringspot virus* or *Peony mosaic virus*, is one of the most widespread viruses of peonies. There have been reports of this virus throughout Asia, Europe, New Zealand, and North America. TRV can infect both herbaceous (*Paeonia lactiflora*) and tree (*Paeonia suffruticosa*) peonies.

Although first described in tobacco, TRV has a wide host range of over 400 species, including: aster (*Aster* spp.), barley (*Hordeum vulgare*), beans (*Phaseolus vulgaris*), beets (*Beta vulgaris*), brassicas (*Brassicaceae*), cocklebur (*Xanthium* spp.), common chickweed (*Stellaria media*), corn (*Zea mays*), cucumber (*Cucumis sativus*), faba beans (*Vicia faba*), gladiolus (*Gladiolus* spp.), iris (*Iris* spp.), lambsquarters (*Chenopodium album*), daffodil (*Narcissus* spp.), oat (*Avena sativa*), onion (*Allium cepa*), peas (*Pisum sativum*), petunia (*Petunia* x *atkinsiana*), pepper (*Capsicum* spp.), pigweed (*Amaranthus* spp.), potato (*Solanum tuberosum*), purslane (*Portulaca oleracea*), rye (*Secale cereale*), shepherd's purse (*Capsella bursa-pastoris*), spinach (*Spinacia oleracea*), sunflower (*Helianthus annuus*), tulip (*Tulipa* spp.), and wheat (*Triticum* spp.). Many of these hosts are symptomless. TRV infection of potato can cause stunting, foliar symptoms (mottling, yellow ringspots or line patterns), stem mottling, as well as tuber deformation and symptoms known as corky ringspot, or spraing (necrotic flecks, arcs, or rings). As such, TRV in potato is of economic importance as corky ringspot may lower the value of a potato shipment, or make it unmarketable. The virus often remains localized to the roots of infected hosts, but in the case of peony and potato may express in the leaves. Some of these hosts can play an important role in the epidemiology of the pathogen on peony.

Tobacco rattle virus particles are rod-shaped and are composed of two single-stranded RNAs. Lengths of both particles are variable, depending on the isolate. RNA-1 is 185 to 196nm long, and RNA-2 is approximately 50 to 115nm long. The diameter of both particles is approximately 23nm. Given that a nanometer (nm) is one-billionth of a meter, these particles are extremely small and can only be seen with an electron microscope. The number of nucleotides is related to particle length, and as such, is variable. RNA-1 is approximately 7000 nucleotides long, and RNA-2 varies from 2000–4500 nucleotides. There are different methods for determining particle length and the number of nucleotides from that relationship. RNA-1 contains all the genes needed for replication and RNA-2 contains the gene necessary to produce the coat protein. The so called "M-type" isolates contain both RNA particles and are nematode and mechanically transmissible. "NM-type" isolates only contain RNA-1, do not form a particle, are not nematode transmissible, and are more difficult to transmit mechanically. Both "M" and "NM" type isolates have been detected in peony. The type of TRV isolate present can impact the ability of the virus to be positively detected, as those isolates lacking a coat protein cannot be detected using antibody-based tests (such as an ELISA) and must be detected using molecular methods (such as PCR).

What are the symptoms of TRV in peony?

TRV in peonies is most commonly expressed as ringspots of alternating green and yellow concentric circles (Figure 1) or a yellow-green mottle or mosaic. Symptoms can also appear as yellow line patterns (Figure 2) or chevrons and symptomatic tissues can turn purple or red in certain conditions (Figure 3, Figure 4). These symptoms can affect marketability of a whole plant or stems if present during flower harvest. There are no known symptoms of TRV expressed in the flower and it is unclear how the virus affects plant productivity; however, observations suggest there is no marked reduction in the vigor of infected plants.

Symptom expression of TRV in peonies, like many viruses, is highly dependent on environmental conditions. Symptoms will often appear during cooler parts of the growing season and are largely absent during the warmer months. Symptoms may also only be apparent in part of the plant while the remainder of the plant appears healthy (Figure 5; Figure 6). Even if symptoms are not visible, if any parts of the plant show or have ever shown symptoms of TRV, it is likely that the entire plant is infected with the virus. It is not possible to remove only infected plant parts or cure a peony of TRV (see "What do I do about my TRV-infected plants?"). Peonies are also susceptible to other viruses, such as *Tomato spotted wilt virus* (TSWV), the symptoms of which can resemble TRV. TSWV can also cause economic damage to peony and many other host species, but requires different management strategies than TRV.

Figure 1. A peony leaf displaying ringspots of alternating yellow and green concentric circles which are characteristic symptoms of Tobacco rattle virus. (Published with permission of Gary A. Chastagner.)

Figure 2. A peony leaf showing yellow line pattern due to infection by Tobacco rattle virus. (Published with permission of Todd Steinlage.)

Figure 3. Peony leaves with purple chevron patterns associated with Tobacco rattle virus. (Published with permission of Andrea R. Garfinkel.)

Figure 4. Purple blotching on a peony leaf due to infection by Tobacco rattle virus. (Published with permission of Andrea R. Garfinkel.)

Figure 5. One peony shoot displaying symptoms of Tobacco rattle virus among others from the same plant and other plants without symptoms. (Published with permission of Gary A. Chastagner).

Figure 6. A single leaflet on a peony plant showing ringspot symptoms of Tobacco rattle virus while other nearby leaves on the same plant appear to be healthy. (Published with permission of Gary A. Chastagner.)

How did TRV get into my field?

If TRV symptoms are present in a first year planting, infected rootstock are the most likely method of introduction. Due to the potentially fleeting nature of TRV symptoms, infected plants can be dug, divided, and sold without the supplier knowing the plant is infected. Each root piece cut from an infected plant will contain the virus; therefore, it is not unreasonable to observe a small percentage of new peony plants infected with TRV (if a large percentage of a new planting is infected with TRV, it may be worth notifying the supplier). Rootstock producers who plant back their own stock may unknowingly be dividing infected plants and increasing the proportion of infected rootstock in their field. Healthy rootstock can also be infected by nematodes that acquire the virus from a previous crop or nearby infected host.

It is also possible for a healthy plant to become infected with the virus by a nematode vector or by mechanical transmission after being planted into a field (see "How does TRV spread?"). TRV does not survive in the soil absent of its nematode vector.

How does TRV spread?

The "M-types" of TRV are transmitted by nematodes in the genera *Paratrichodorus* and *Trichodorus* (multiple species, such as *P. allius*, *P. anemones*, *P. minor*, *P. nanus*, *P. pachydermus*, *P. teres*, *T. cylindricus*, *T. primitivus*, *T. similis*, and *T. viruliferous*), known collectively as stubby root nematodes. Trichodorid nematodes are migratory ectoparasites (live outside the plant) and feed with a toothlike (or needlelike) stylet. The nematodes pick up the virus from an infected plant by feeding on the roots and then can transmit the virus to healthy plants through subsequent feeding activity. Both adults and juveniles can transmit TRV. Once the plant becomes infected, the virus multiplies and spreads throughout the plant, or may in some cases be restricted primarily to the roots. Besides being able to transmit the virus, these nematodes do not generally cause damage to the peony. Stubby root nematodes are highly mobile throughout the soil profile and may be found from the surface to depths below 40 inches. It is unclear how far they will travel horizontally, but it is thought their movement across a field without tillage or other soil disturbance is relatively slow. Completion of their lifecycle may take greater than six weeks in cold soils. The nematodes are favored by abundant soil moisture, and have difficulty penetrating densely packed soils with high clay, silt, or very fine sand particles (<50µm). "NM-type" isolates have not been shown to be able to be vectored by nematodes.

The nematode vector is not present in all peony production regions. Fields throughout Alaska were surveyed in 2013, 2014, and 2015 by the Alaska Division of Agriculture for nematodes, but stubby root nematodes were not found. Stubby root nematodes have been reported in potato fields in Washington, Oregon, and Idaho but studies have not been conducted specifically in peony fields.

TRV is also transmissible by sap under experimental conditions; however, the efficiency of this type of mechanical transmission on contaminated tools is not documented in peony. It is thought that the "M-type" TRV isolates are more stable in sap than "NM-type" isolates. TRV is not thought to spread from plant to plant by touching. Maintenance in a field between seasons may occur through the seed of some weed hosts, such as *Viola arvensis*, *Stellaria media*, and *Capsella bursa-pastoris*, but this is only relevant in the epidemiology of TRV in peony in areas where the vector is present.

What do I do about TRV-infected plants?

Peonies cannot be cured of TRV; management options focus on preventing healthy plants from becoming infected through a combination of removing infected plants and reducing the potential for spread of the virus.

Management in cut flower operations may be different than those in rootstock production fields. In rootstock production fields, any plants with virus symptoms should be removed (rogued) to prevent division and sale of infected plant material. Removal of infected plants also prevents spread of the virus from adjacent plants via vector or mechanical transmission.

Rootstock producers may also take care to remove all soil from their product to ensure that stubby root nematodes are not being introduced in infested soil. This is especially true if rootstocks are being sold to and planted in areas where the nematodes are not known to be present.

In a cut flower operation, growers may choose to rogue plants, yet others may choose to attempt to manage the spread of the virus without removing the plant. This is especially true in areas where the nematode vectors are not known to be present, such as in Alaska. Although the efficiency and mechanisms for mechanical transmission of TRV in peonies is not well understood, efforts to prevent mechanical transmission by sanitation of hand tools should be practiced. There is no information currently available on managing the mechanical spread of TRV. However, understanding transmission of closely related viruses may give us insight into TRV management.

TRV is in the same virus family as *Tobacco mosaic virus* (TMV) (Family *Virgaviridae*) and, as such, it is possible that it will have similar responses to disinfection. Ideally, tools are disinfected between plants; disinfection after working a symptomatic (or previously symptomatic) plant is highly recommended. Some growers use multiple tools, so that one is soaking in disinfectant while the other is in use. Tools should first be wiped clean of excess sap or debris. Several disinfectants are available, each with its own requirements.

A 1:21 chlorine bleach solution (1 part bleach to 21 parts water) can be applied to tools for a one-minute exposure. Bleach solutions lose effectiveness after a few hours, are corrosive, and may damage eyes, skin, and clothing. A 2% solution of potassium peroxymonosulfate and sodium chloride (e.g., Virkon S) also requires one minute of soaking and is corrosive, but maintains effectiveness for about one week. Products combining hydrogen dioxide (hydrogen peroxide) and peroxyacetic acid (e.g., Oxidate or ZeroTol) require soak periods of 1–5 minutes, and are corrosive. Quaternary ammonium products (e.g., Green-Shield or KleenGrow) generally require longer soak periods (up to 10 minutes), and are corrosive. Pine oil products can also be used, generally requiring 3 to 10 minutes soak time. Tools should be rinsed following all disinfectants. By law, all products must be used according to their label directions (note that it is permissible to use products at a lower concentration or lower application frequency than labeled, unless specifically prohibited). Some pesticides mentioned here may not be registered for homeowner use. Readers are advised to check all applicable regulations in the state in which use will take place.

If the nematode vector is present, removal of infected plants is the only way to reduce spread of TRV. Vector control by fumigation has not been successful for many crops due to the deep distribution and high vertical mobility of the nematode.

It would be advisable to grow plants in soil that is vector-free, and take measures to exclude vector and virus introduction. However, some vector nematode species have wide host ranges, including cultivated and weedy species. Therefore, in both cut flower and rootstock production systems, removal of weedy hosts can reduce reservoirs of TRV and nematodes.

If there is question of whether the nematode vector is present in your field, soil tests are available to identify stubby root nematodes. See below for soil testing locations.

TRV does not survive in the soil absent of its nematode vector; therefore, the virus will not spread to a healthy plant that is used to replant a site where an infected plant is removed.

As mentioned in the “How Does TRV Spread?” section, the strain of the virus present may impact management decisions. Since the “NM-type” isolates are less likely to be vectored by nematodes or mechanically transmitted, removal of infected plants or tool sterilization may not be necessary if this is the only strain present in the field. Whether the “M-” or “NM-type” is present can be determined by a series of tests, but most diagnostic clinics do not routinely determine the virus strain during diagnostic activities.

Where can I send my peonies for virus testing?

Virus infections can be positively identified by using either antibody-based (ELISA) or molecular (PCR) tests that can be performed by various private and public labs. When testing for TRV, a PCR-based test is preferred because an ELISA cannot detect “NM-type” TRV isolates. Testing for multiple potential viruses is advised when sending in plant material for virus indexing because of the similarity of other virus symptoms to those of TRV. The age and the quality of plant tissue can affect the ability to detect the presence of the virus; contact the testing lab about how to collect plant tissue for best results. Given the difficulty of positive identification of the virus, a negative test result should be interpreted with caution. It may be necessary to send in samples during multiple times of the year or subsequent years from suspected infected plants to get a positive identification of the virus.

Most plant clinics can either test or arrange to have samples tested for TRV. The cost of testing varies between labs; contact labs directly to find associated testing charges.

In the Pacific Northwest, the following university labs accept samples for virus testing:

Oregon State University Plant Clinic, Hermiston

Website: http://oregonstate.edu/dept/hermiston/plant-pathology-plant-lab-testing
Contact Person: Robert Cating
Phone: 541-567-8321
Accepts out-of-state samples: Yes

Oregon State University Plant Clinic, Corvallis

Website: http://plant-clinic.bpp.oregonstate.edu/
Contact Person: Melodie Putnam
Phone: 541-737-3472
Accepts out-of-state samples: Yes

Washington State University, Clean Plant Center Northwest

Website: http://cpcnw.wsu.edu/virus_lab/
Contact Person: Tina Vasile
Phone: 509-786-9382
Accepts out-of-state samples: Yes

Washington State University Plant Clinic, Puyallup

Website: https://puyallup.wsu.edu/plantclinic/
Contact Person: Jenny Glass
Phone: 253-445-4582
Accepts out-of-state samples: No

Washington State University Plant Clinic, Pullman

Website: http://plantpath.wsu.edu/diagnostics/
Contact Person: Rachel Bomberger
Phone: 509-335-3292
Accepts out-of-state samples: Yes

In Alaska, samples can be sent to:

Alaska Department of Natural Resources

Alaska Plant Materials Center
Website: http://plants.alaska.gov/PathologyForms.html
Contact Person: Todd Steinlage
Phone: 907-745-8138
Email: Todd.Steinlage@alaska.gov
Accepts out-of-state samples: No

It may also be desirable to test the soil for the presence of stubby root nematodes.

The following labs accept soil samples for nematode analysis:

Oregon State University Nematode Testing Service, Corvallis

Website: http://plant-clinic.bpp.oregonstate.edu/nematodes
Contact Person: Nadine Wade
Phone: 541-737-5253
Accepts out-of-state samples: Yes

University of Nebraska-Lincoln Nematology Laboratory

Website: http://nematode.unl.edu/diagnostics.htm
Contact Person: Lisa Sutton
Phone: 402-472-5770
Accepts out-of-state samples: Yes

University of Idaho

Parma Research and Extension Center
Website: https://www.uidaho.edu/cals/parma-research-and-extension-center
Contact Person: Dr. Saad L. Hafez
Phone: 208-722-6701
Accepts out-of-state samples: Yes

Resources

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