Managing rusty root disease: A survey shows that the fungus causing rusty root is common in stratified seed.

Richard Reeleder
reelederr@agr.gc.ca
519-457-1470 x297

AAFC
1391 Sandford St.
London ON N5V 4T3

Summary (August 2007 update):
Previously, we showed that *Rhexocercosporidium panacis* is the cause of rusted root (rusty root) of ginseng (Phytopathology 96:1243-1254, 2006). As detection and elimination of inoculum sources is often a successful approach in disease management, in late summer and fall 2006, we carried out a survey to locate possible sources of inoculum of this disease. Using a molecular method, we tested samples of soil, straw mulch and seed for presence of the fungus. The fungus was detected rarely in soils not previously used for ginseng and in 'new' straw. By contrast, the fungus was frequently detected in four-year-old ginseng gardens and in weathered straw collected from raised beds in these gardens. One of 12 lots of 'green' (non-stratified) seed contained detectable populations of *R. panacis;* however, no more than 1 of three sub-samples from this green seed lot was positive for the fungus. By contrast, 9 of 15 lots of stratified seed contained the pathogen. We conclude that stratified seed is a major source of inoculum dispersal and introduction into ginseng gardens.

Introduction:
In earlier work, funded in part by the OGGA, a new species of fungus, *Rhexocercosporidium panacis*, was found in diseased 'rusty root' tissue but not in healthy root tissue. The ability to reproduce symptoms on ginseng roots was confirmed in pathogenicity tests. Oligonucleotide primers based on ITS sequences were designed to amplify DNA of *Rhexocercosporidium* spp. Polymerase chain reaction assays on DNA extracted from naturally infected root tissue showed that the fungus was present in nearly all rusty roots (>95% of Ontario roots with these disease symptoms) but was infrequent (6%) in healthy appearing roots. Two *Fusarium* spp previously associated with rusty root were much less frequent (e.g. 12% detection rate for *F. equiseti* in Ontario roots; 0% for *F. culmorum*) and were absent from a large percentage of diseased roots. The most probable cause of rusted root of ginseng is therefore a previously undescribed species of *Rhexocercosporidium,* now named *R. panacis*. In-vitro assays suggested that several fungicides might be effective in controlling the disease. See Phytopathology 96:1243-1254 and Final Report of the MII A06101 Dec. 2006 for further details.

Detection and elimination of inoculum sources is often a successful approach in disease management. Once the source of the disease is located, modifications in grower practices might permit the inoculum to be eradicated at little or no cost. If successful, this approach might be both more effective and less costly than other approaches, such as fungicide application.

2006 survey:
Soils: Samples were collected using disinfected soil probes from four sites where ginseng had not been grown previously. Two of these sites were located at AAFC-Delhi and two at OMAF-Simcoe. Four samples (each consisting of several combined soil cores) were taken from different locations within each site. Five growers allowed us to sample from two and/or four-year-old ginseng gardens. Four different sites within each garden were sampled.

Mulch: Samples were collected from straw bales at five different sites, with four being taken from each bale. Samples were also collected from weathered mulch in two and four-year-old gardens. These were taken from the same locations as the corresponding soil samples (as above).

Seed: Four growers allowed us to collect berries from different fields, resulting in 12 samples. Berries were mechanically-depulped as soon as feasible at AAFC-Delhi. The resulting 12 green seedlots were then prepared for DNA extraction. Several lots of stratified seed were provided by growers.

DNA extraction: DNA was extracted from soil samples using the method developed for Cylindrocarpon (Plant Pathology 56: 508-516, 2007). Straw mulch and seed samples were freeze-dried then ball-milled prior to DNA extraction. In total, approx 200 samples were extracted. The extracts were then tested for the presence of *Rhexocercosporidium* by amplifying the target DNA in two rounds of polymerase-chain-reaction. The final products were examined electrophoretically in order to determine if the pathogen's DNA was present in the extract.

Results: The fungus was detected in one of four 'non-ginseng' soils. At that location, no more than one of four subsamples were positive for the fungus, suggesting that the fungus has a limited distribution in soil. The fungus was detected with increasing frequency in two and four-year-old ginseng crops. In four-year-old gardens, all six gardens had detectable populations; in four of these, 3-4 of 4 subsamples were positive, suggesting that the populations were quite high or well-distributed. However, in two gardens, apparently lower populations were detected; this might indicate that differences in grower practice, seed quality, or soil affect the incidence of rusted root.

One of four sites where 'new' straw was collected had a detectable population in the straw, but in that case, only 1 of 4 subsamples was positive. When weathered mulch from 4-yr-old gardens was assayed, 5 of six sites were positive for the fungus. Subsample detection levels in these mulch samples mirrored detection in the soils from the same gardens.

One of 12 green seed samples was positive for *R. panacis*, and in these cases just one of four subsamples was positive. By contrast, 9 of 15 lots of stratified seed contained the pathogen.

Conclusions: Established gardens and stratified seeds both appear to be significant sources of inoculum that could affect new plantings. Stratified seed appears to be the most likely source of introduction of the fungus into new fields.

Note: The fungus Rhexocercosporidium panacis was detected using an experimental method. In cases where results for a sample were negative, this indicates that DNA of fungus was not present at the level of detection for the method. In some cases, a more sensitive method may have given a positive result. Differences in detection may be affected by sample collection procedures, impurities in extracts and other factors. **Failure to detect the fungus is therefore not a guarantee that the sample did not contain the fungus**. See additional notes in Tables.