Sources of inoculum of *Rhexocercosporidium panacis*, the cause of rusted root of ginseng.

Abstract:

Detection and elimination of inoculum sources is often a successful approach in disease management. A cultivation-independent technique was used to determine possible sources of inoculum of *Rhexocercosporidium panacis,* the cause of rusted root (rusty root) of ginseng (Phytopathology 96:1243-1254; Mycologia 99 (in press)). Nested PCR amplifications were used to detect inoculum in soil, straw mulch and seed samples. DNA was first extracted from soil (Can. J. Plant Pathol. 25:182-191) and plant samples. DNA was then amplified using, first, a universal ITS5/ITS4 primer set, followed by amplification with the *Rhexocercosporidium*-specific ONBCU3 [5'-CAAAGAATAGACAGCGCCTCACAT-3'] / ONBCL2 [5'-CCCCCGGAATACCAGAG-3'] oligonucleotides. To confirm identity of PCR products, DNA from representative bands observed in electrophoretic gels was purified and compared to GenBank sequences. 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. However, in certain older gardens and weathered straw samples, the fungus was rarely detected. One of 12 lots of 'green' (non-stratified) seed contained detectable populations of *R. panacis*. 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:

Rusted root (rusty root) of ginseng is a common disease throughout the ginseng growing regions of North America. The dominant symptoms include superficial lesions that give the root a scabby appearance; affected roots have reduced market value (Fig 1). The cause of the disease was only recently established as the fungus *Rhexocercosporidium panacis* (Reeleder 2007; Reeleder et al 2006).

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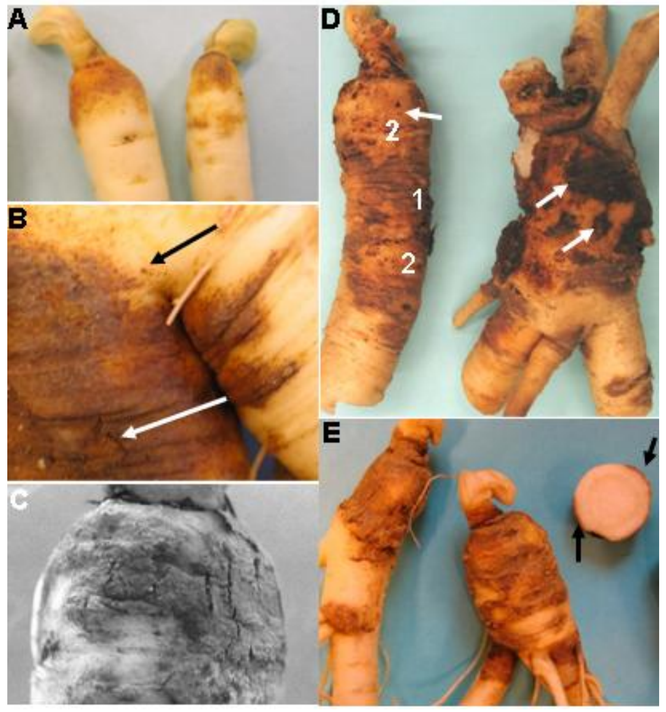

Fig. 1. Symptoms of rusted root observed on field-collected roots. A. Crown lesions on 1-yr-old roots. B. Lesion on 5-yr-old root with small "blister" lesions (black arrow) and initial rupturing of periderm (white arrow). C. Lesion with rupturing periderm tissue. D. Severe rusted root on 4-yr-old roots. Arrows indicate blackened remnants of periderm. "1" indicates areas where periderm is discolored but intact; "2" indicates areas where periderm has been rotted away. E. Severe rusted root on 3-yr-old roots. Arrows indicate location of lesions.

Table 1. Detection of *R. panacis* in soil and mulch from two and four-year-old ginseng gardens.
Number of positives per 4 sub-samples are reported.

grower	1	2	3	4	5	6
2 yr ginseng soil	1/4	1/4	0/4	4/4		
2 yr ginseng mulch	2/4	0/4	0/4	0/4		
4 yr ginseng soil	1/4	4/4	2/4	4/4	3/4	4/4
4 yr ginseng mulch	1/4	4/4	0/4	4/4	2/4	4/4

Table 2. Detection of *R. panacis* in fresh ('green') seed lots and stratified seed lots.
Number of positives per 3 sub-samples are reported.

Seed lot:	1	2	3	4	5	6	7	8	9
Green	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
Stratified	3/3	0/3	0/3	2/3	1/3	3/3	3/3	1/3	1/3

Seed lot:	10	11	12	13	14	15		
Green	0/3	0/3	0/3				Number lots contaminated	1/12
Stratified	1/3	0/3	0/3	0/3	2/3	0/3	Number lots contaminated	9/15

Methods:

Samples (soil, straw mulch, seed)

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Freeze-drying

Milling

DNA extraction

PCR: two-stage (nested)

Fig. 2. Outline of methods used. Soil samples were extracted as previously described (Reeleder et al 2003). Straw mulch and seed samples were freeze-dried to stabilize DNA, then ball-milled to reduce particle size. DNA was extracted from straw mulch using an SDS extraction method; DNA from seeds was obtained using a CTAB method. Products of nested PCR were subject to electrophoresis; ethidium bromide staining was used to detect products.

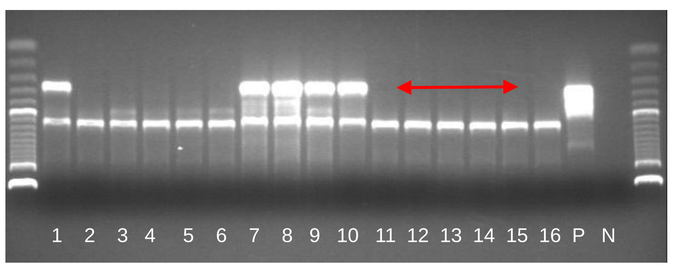

Fig. 3. The above gel image represent results of a seed assay. Red arrow indicates samples are positive (375 bp). Non-375bp bands are products of ITS5/ITS4 amplification (1st round). Lanes 1-10 are extracts from stratified seed; lanes 16 are extracts from green seed. P=positive control; N=negative control.

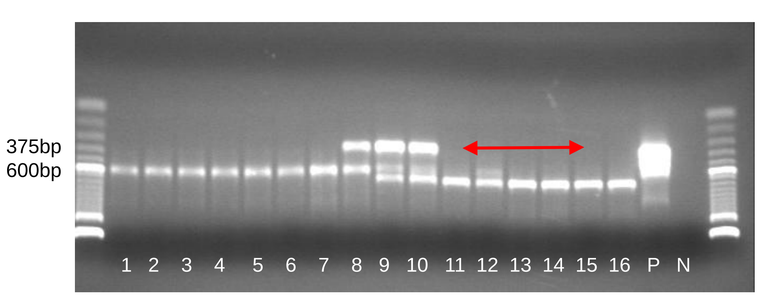

Fig. 4. The above gel image represent results of straw mulch and seed assay. Red arrow indicates samples are positive (375 bp). Non-375bp bands are products of ITS5/ITS4 amplification (1st round). 600bp bands may represent other fungi or plant material; 650bp bands are ITS5/ITS4 *R. panacis* products; 750bp bands represent Panax DNA. Lanes 1-8 are new straw extracts; lanes 9-12 are stratified seed; lanes 13-16 are fresh (green) seed. P=positive control N=negative control.

Conclusions:

- Stratified seed is often contaminated with *Rhexocercosporidium*.
- The fungus is common in old ginseng fields and old straw mulch.
- *R. panacis* is rare or absent in green seed.
- The fungus is rare or absent in 'new' fields and 'new' straw.

Some things we don't know:

- Will disruption of seed inoculum source control disease?
- How does seed get contaminated?
- How does disease spread in the field?
- Are there correlations of soil populations and DI or DS?
- Can the fungus be detected on dried roots?
- Are there efficacious fungicides?

References:

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