## Appendix A. Soilborne Pathogens and Root Diseases

No disease or pathogen measurements were made over the course of these rotation studies at the farmer sites. However, in Fall 2003, soil samples were taken from the rotations at the end of the experiment at the farms near Colfax, Dusty, and Lamont. These samples were sent to Australia for DNA analysis by SARDI (South Australian Research and Development Institute) (Ophel-Keller 2003). This test provides a quantitative analysis of the pathogen populations in the soil, based on picograms DNA/g of soil. With this method, DNA is extracted from soil, and PCR primers specific to each pathogen are used to amplify the DNA. The following pathogens are detected by this test: *Gaeumannomyces graminis* var. *tritici* (take-all), *Rhizoctonia solani* AG8, (*Rhizoctonia* root rot), *Heterodera avenae* (cereal cyst nematode), *Pratylenchus neglectus*, and *P. thornei* (lesion nematode); *Fusarium pseudograminearum* and *F. culmorum* (Fusarium crown rot) and *Bipolaris sorokiniana* (common root rot).

We hypothesized that root diseases result from inoculum in the soil and residue, which are produced during the previous year(s) of cropping. These pathogens produce resistant spores (*Fusarium, Bipolaris*) or survive as mycelium in decaying roots (*Rhizoctonia, Gaeumannomyces*). This test also detects 3 important nematodes: cereal cyst nematode (*Heterodera*) and 2 species of lesion nematode (*Pratylenchus*).

It is well known that crop rotation will influence certain root pathogens, if one of the crops is a non-host or if there is no crop (fallow). During this unfavorable period for the pathogen, inoculum in the soil will decline over time, due to microbial attack, feeding by soil invertebrates, or loss of energy through respiration. In any case, the inoculum level of certain pathogens will be reduced following crop rotation and fallow, and we hypothesized this test would detect these differences.

### Materials and Methods

Soil samples were taken in Fall 2003 from fields that had been in various rotations since 1997. There were a number of different rotations:

4-year - the standard Beck rotation - SW-WW-corn-spring broadleaf

4-year - reverse - WW-SW-corn-spring broadleaf

3-year standard - WW-SB-chemical fallow

3-year alternate - WW-SB-spring broadleaf

3-year mixed - WW-SB-chemical fallow or spring broadleaf

Because of the unbalanced design, not every rotation was sampled equally. Table 1 shows the number of samples from each rotation at each location.

Table A1. Number of samples from each rotation.

| Site | 4-yr | 4-yr reverse | 3-yr standard | 3-yr alternate | 3-yr mixed |
|---|---|---|---|---|---|
| Colfax | 4 | 0 | 1 | 0 | 2 |
| Dusty | 4 | 4 | 3 | 0 | 0 |
| Lamont | 3 | 3 | 2 | 2 | 2 |

For each sample, approximately 1 kg of soil was dug from the upper 6 inches. Soil was air-dried and frozen at -20°C for 2 weeks before being sent to Australia. The freezing was a quarantine requirement to kill any insects in the soil. Results were received in a spreadsheet as picograms DNA/g soil. DNA levels were log transformed after adding 1 to each of the values, in order to make the data normally distributed. ANOVA was performed using rotation and strips as main factors. Means were separated with LSD at $P$=0.05. If data was not normally distributed after transformation, means were separated with Kruskal-Wallis One-Way non-parametric ANOVA. Risk levels were also assigned to the values, based on Australian conditions, but may not be applicable to our conditions.

Because of the unbalanced designs and the lack of replicates of some rotations, the following comparisons were made:

1. At the Colfax and Lamont sites, the standard 3-year rotation was compared with the mixed and alternate 3-year rotations.

2. At the Dusty and Lamont sites, the standard 4-year rotation was compared with the reverse 4-year rotation.

3. All 4-year rotations were compared with all 3-yr rotations, using data from all 3 sites.

Only *Rhizoctonia solani, Fusarium culmorum,* and *Fusarium pseudograminearum* were analyzed statistically, since the level of the other pathogens were low or below the detection limit.

**Results and Discussion**

The take-all pathogen, *Gaumannomyces graminis* var *tritici*, was below detectable levels in all samples taken. This pathogen is normally not a problem in the normal 3-year or 4-year rotations in eastern Washington that contain a non-host broadleaf crop (peas, chickpeas, lentils, canola, sunflower, safflower, buckwheat, flax), a fallow, or a non-host monocot (corn, oats, millet). This data fits the observations of numerous studies on this disease over the past 50 years.

The cereal cyst nematode was not detected in any samples. This nematode is a problem in northeast Oregon and the Willamette Valley, but has not been detected in the dryland areas of Washington. *Pratylenchus neglectus*, the root lesion nematode, was only detected in 5 out of 7 samples in Colfax, but in 10 out of 11 samples from Dusty. In Lamont, only 1 out of 14 samples contained *P. neglectus*. *P. thornei*, the more virulent of the 2 species, was found in only 1 sample.

These nematodes have been implicated in yield losses on cereals in Oregon (Smiley, personal communication and submitted manuscripts). They have a wide host range, with wheat being the most preferred host, followed by barley. They can also reproduce on broadleaf crops, but there is variability among species and cultivars in terms of reproductive success.

*Rhizoctonia solani* was detected at all 3 sites. In Colfax, it was detected in 3 out of 7 samples, at a high level in 1 and a medium level in 1. In Dusty, *Rhizoctonia* was detected in 4 out of 11 samples, and in 10 out of 14 samples in Lamont. At Lamont, 4 of the positive samples had a medium level and 1 had a high level of DNA.

*Fusarium pseudograminearum* was predominant over *F. culmorum* in all sites, similar to other data from low and intermediate rainfall areas. In Colfax, 5 out of 7 samples contained *F. pseudograminearum,* with levels ranging from low to high. In Dusty, 6 out of 11 samples contained *F. pseudograminearum,* but only 3 out of 14 samples were positive at Lamont.

*Bipolaris sorokiniana* was below the detection limit in all samples, except for 1 in Colfax.

**Comparison of 3-year rotations**

There were no significant differences between the Colfax and Lamont sites, and no interactions between site and rotation, so the results were pooled among the 2 sites (Table 2). There was no significant effect of the type of 3-year rotations on the DNA levels of *Rhizoctonia* or *Fusarium.* This suggests there is no difference between chemical fallow and a spring broadleaf rotation crop. This makes sense for *Fusarium*, since the spring broadleaf crop should not be host for these pathogens. However, *R. solani* can also infect broadleaf rotation crops, so these results do not fit our theory.

Table A2. Comparison of 3-year rotations.

| Rotation | *Rhizoctonia solani* [log (pg DNA/g soil +1)] | *Fusarium culmorum* [log (pg DNA/g soil +1)] | *Fusarium pseudograminearum* [log (pg DNA/g soil +1)] |
|---|---|---|---|
| 3-yr standard | 0.58 | 0.07 | 0.07 |
| 3-yr mixed | 0.89 | 0.47 | 1.42 |
| 3-yr alternate | ND | ND | ND |
| P | NS (0.31) | NS (0.59) | NS (0.13) |

ND = not determined, not enough degrees of freedom.

**Comparison of 4-year rotations**

There were no significant differences between the Dusty and Lamont sites, and no interactions between site and rotation, so the results were pooled among the 2 sites (Table 3). There was no significant effect of the type of 4-year rotations on the DNA levels of *Rhizoctonia* or *Fusarium.* This would be expected, since spring and winter wheat are both susceptible to these pathogens, and reversing the order of the cereals should not create a difference.

Table A3. Comparison of 4-year rotations.

| Rotation | *Rhizoctonia solani* [log (pg DNA/g soil +1)] | *Fusarium culmorum* [log (pg DNA/g soil +1)] | *Fusarium pseudograminearum* [log (pg DNA/g soil +1)] |
|---|---|---|---|
| 4-yr standard | 0.80 | 0.27 | 0.63 |
| 4-yr reverse | 1.01 | 0.34 | 0.41 |
| P | NS (0.56) | NS (0.74) | NS (0.38) |

**Comparison of 3-year with 4-year rotations**

There were no significant interactions between site and rotation, so the results were pooled among the 3 sites (Table 4). There were no significant differences among sites, except for higher levels of *F. pseudograminearum* at the Colfax, compared with the other sites. *Rhizoctonia solani* and *F. pseudograminearum* did not show any rotation effect. However, there were higher levels of *F. culmorum* in the 4-year rotation, compared with the 3-year rotation. But, for *F. culmorum,* the interaction between site and rotation was close to significant (0.06), and a closer examination of the data showed this rotation effect was most pronounced at the Colfax site, but was not very pronounced at the Dusty site, while the trend was reversed at the Lamont site.

A general conclusion is that including a warm-season grass in the rotation does not affect the level of these 3 pathogens. *Rhizoctonia solani* can also infect corn and millet, although it may be a different AG type than the AG-8 detected here. *F. culmorum* also infects both corn and millet. The host range of *F. pseudograminearum* on these warm-season grasses is not really known. This species was previously grouped with *F. graminearum*, which is a well-known pathogen on corn and millet. But, given the ability of these 3 pathogens to also infect the warm-season grass, a 4-year rotation would not give any additional rotation benefit.

Table A4. Comparison of 3-year with 4-year rotations.

| Rotation | *Rhizoctonia solani* [log (pg DNA/g soil +1)] | *Fusarium culmorum* [log (pg DNA/g soil +1)] | *Fusarium pseudograminearum* [log (pg DNA/g soil +1)] |
|---|---|---|---|
| 3-yr | 0.80 | 0.21 | 0.77 |
| 4-yr | 0.83 | 0.58 | 0.83 |
| P | NS (0.92) | 0.05 | NS (0.84) |

**General Conclusions**

Based on the DNA results, the risk of most soilborne pathogens at these sites was rather low. The only exception was the level of *Rhizoctonia* at the Lamont site, which was higher than the other sites. This agrees with my sampling and previous observations at these farms, as part of other research projects. We have often seen patching and uneven stands in the Lamont area, indicative of *Rhizoctonia,* but we have not seen these symptoms at the Colfax site. At the Colfax site, the major pathogen was *F. pseudograminearum* and was in high enough levels to probably cause yield losses in low-rainfall years. In general, however, including a warm-season grass in the rotation does not affect the level of these 3 pathogens, because these crops (corn and millet) also support these pathogens.

## References

Ophel-Keller, K., McKay, A., Hartley, D., Driver, F., Wanjura, W., Heap, J., Herdina, Dumitrescu, I., and Curran, J. 2003. Quantitative detection of soil-borne plant pathogens. 8th International Congress of Plant Pathology, Christchurch, New Zealand. Vol. 1, p. 122 (abstract).